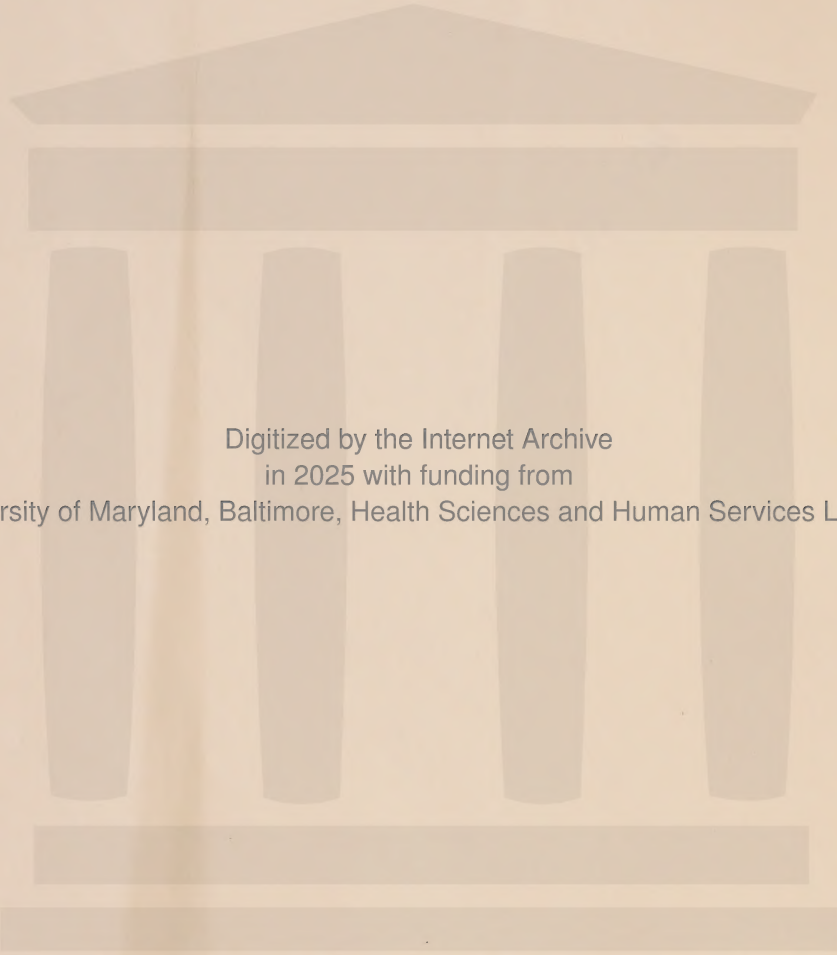


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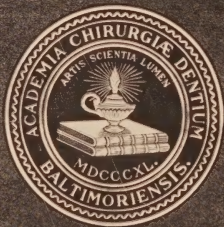
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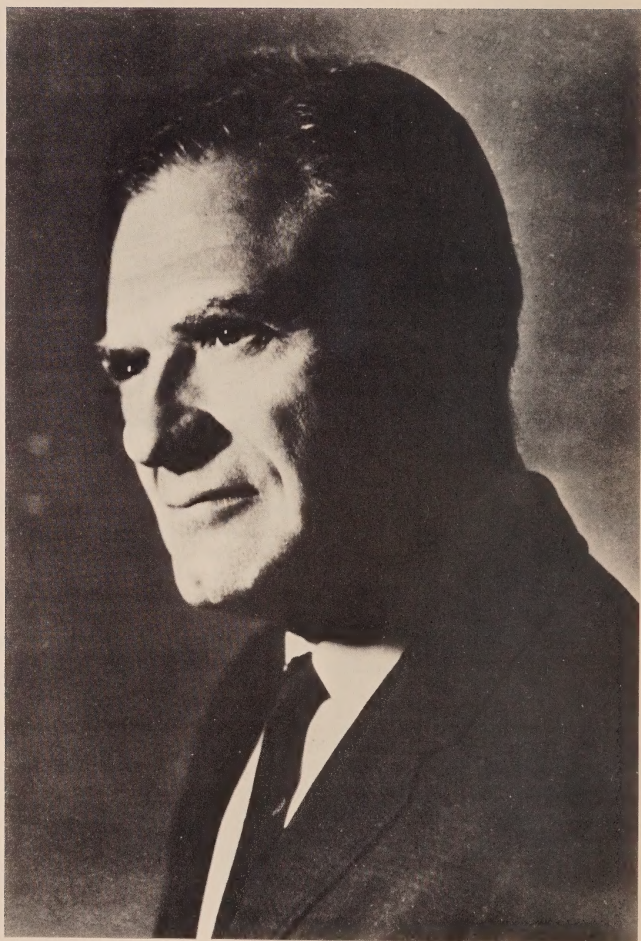
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ARNOLD M. SELIGMAN, M.D.
1912 - 1976

In Memoriam

ARNOLD M. SELIGMAN, M.D. 1912-1976

Dr. Arnold M. Seligman, a surgeon by training, had his greatest impact on the medical profession through his distinguished research in histochemistry and more recently in oncology. Dr. Seligman was on the editorial boards of numerous journals, including *The Journal of Histochemistry and Cytochemistry*, *Journal of Biochemical and Biophysical Cytology*, *Annales d'Histochimie*, *Histochemistry*, *Journal of Electron Microscopy* and *The Journal of the Baltimore College of Dental Surgery*. His research in establishing new methods of intracellular localization of various enzymes brought him numerous awards and worldwide recognition, such as the presidency of The Third International Congress for Histochemistry and Cytochemistry, Maryland Chemist of the Year Award and one of the few lifetime Cancer Grant Awards. Dr. Seligman was also Professor of Surgery at The Johns Hopkins University School of Medicine and Chief of the Research Oncology and Cell Biology Unit at Sinai Hospital at Baltimore.

On a more personal note, it was reassuring for some of us that he was only a telephone call away, in that he was never too busy and was always available for assistance and a friendly chat. The profession lost a giant and many of us lost a valued colleague and friend.

LESLIE P. GARTNER, PH.D.
Associate Professor
Department of Anatomy
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**Program and Performance Evaluations:
1975 Graduates of
the University of Maryland Dental School
Baltimore College of Dental Surgery**

ROBIN H. MISSLER, M.S.W.
JOHN F. HASLER, D.D.S., M.S.D.
MARK L. WAGNER, D.M.D.

Program and Performance Evaluations: 1975 Graduates of the University of Maryland Dental School Baltimore College of Dental Surgery

ROBIN H. MISSLER, M.S.W.*
JOHN F. HASLER, D.D.S., M.S.D.†
MARK L. WAGNER, D.M.D.‡

In April, 1976, the Dental School of the University of Maryland conducted an exploratory study for purposes of: 1) determining the effectiveness of the academic preparation of its graduates who had entered postgraduate training, associateships, and military duty; 2) strengthening the present curriculum by identifying potential strengths and weaknesses; 3) gathering information that would be helpful in counseling future students regarding career pursuits, and 4) establishing rapport with existing program directors and dentists in the community for future student placements. This is the first time, to our knowledge, that a formalized study of this nature has been conducted at the Dental School.

METHOD

This study was carried out through the Office of the Associate

Dean for Clinical Affairs, the Department of Oral Health Care Delivery, and the Office of Student Affairs by means of a two-section mailed questionnaire. Fifty-five graduates (Group I) from the Class of 1975 who had indicated their professional activities (See Table 1) for 1975-1976 included advanced training, associateships or military duty were surveyed. Simultaneously, the 55 program directors and associates of these same graduates were surveyed (Group II).

Each graduate surveyed was asked to complete the Group I questionnaire, evaluating both their undergraduate dental school training and their present program/associateship experience. Program directors/associates were asked to complete the Group II questionnaire, evaluating our graduates under their direction.

*Research Associate, Department of Oral Health Care Delivery.

†Associate Dean for Clinical Affairs.

‡Director of Student Affairs.

DESIGN

Postgraduate Training Evaluation *—Group I: Recent Graduates*

In this questionnaire, 1975 graduates were asked to evaluate the relevance of their undergraduate dental school training to their current daily performance in some of the following areas: Quality and content of Dental School lectures; quality of clinical instruction; correlation of basic sciences to clinical skills; oral examination techniques; treatment plan concepts; plaque control education; concepts of occlusion, operative dentistry, removable prosthodontics, periodontics, endodontics, pediatric dentistry, orthodontics, oral surgery, and dental auxiliary utilization.

In addition, suggestions for improvements in both the clinical and didactic areas of the curriculum were solicited.

Another question asked the graduates to rate the overall quality of their dental education. (See Table 2.)

The second half of the Postgraduate Training Evaluation consisted of asking graduates to evaluate their present program/training experience in areas of: Work Conditions, Research, Patient Care, Supervision, Continuing Education, Responsibility and Financial Support, along with their future career plans (See Table 3) and general comments.

Program Director/Associates *Evaluation Form—Group II*

The Program Director/ Associates' Evaluation Form asked the evaluators to assess their University of Maryland graduate(s) on

level of achievement in the following categories:

- 1) Didactic
 - a. Knowledge of subject
 - b. Initiative, industry and interest
 - c. Ability to use logic and reason
 - d. Ability to carry out search of literature, research, etc.
 - e. Self-enrichment, i.e., elective reading, etc.
- 2) Clinical:
 - a. Correlation of basic science to clinical science
 - b. Knowledge of treatment planning
 - c. Accuracy of diagnosis
 - d. Patient awareness/preparedness, i.e. scheduling, management, neatness, concern for patient
 - e. Professional judgment: i.e. dependability, ability to follow directions
 - f. Quality of clinical skills
 - g. Patient management skills
 - h. General level of preparedness
- 3) Ability to communicate
- 4) Ability to cooperate
- 5) Professional attitude
- 6) Graduate's strengths
- 7) Graduate's weaknesses
- 8) Comments

RESULTS

The response rate of 76% (42 of 55) for the graduates and 84% (46 of 55) for the program directors/associates was high for a mail-out survey.

Table 1 presents a categorization of the responding graduates in the different types of programs:

TABLE 1
Program Participation

Program	Number	Percent
Postgraduate Program (Specialty)	4	10
Residency	17	40
Military	15	36
Associateship	6	14
Total	42	100

When asked to rate the overall quality of their dental education at the University of Maryland (Table 2), graduates responded as follows:

TABLE 2
Graduates' Evaluation of the Quality of Their Undergraduate Education

Category	Number	Percent
Excellent	16	38
Good	24	57
Fair	2	5
Poor	0	0
Total	42	100

These results suggest that most of the 1975 graduates responding felt that their training in dental school was good to excellent.

The graduates' future career plans, when classified by program type are presented in Table 3:

TABLE 3
Future Career Plans of Program Participants

Program	Total Number	Career Plans	Number
Postgraduate Programs	4	Continued limited practice experience	4
Residency Programs	17	General Practice	12
		Advanced Study	4
		Dental Education & general practice	1
Military	15	General Practice	10
		Advanced Study	3
		Undecided	1
		No Response	1
Associateship	6	Continued general practice experience	6
Total	42	Total	42

Comments by the graduates were generally positive in attitude toward their dental education. One graduate stated,

"The most important comment I can make is that my opinion has changed since graduation. Before I got out of the University of Maryland, I felt the school was second rate. Having been in an area where very few alumni are practicing, I can see that the quality of Maryland's Dental School is superior to many other schools on the East Coast."

Another graduate said,

"After being out of school for approximately one year, I have had the opportunity to talk with recent graduates from other schools in detail about their requirements and education. In addition, I have been able to compare their apparent understanding/knowledge of dental procedures and their experiences to

my own. I have found that I was better prepared from a clinical and didactic standpoint than most of those I have talked with. I am very confident and proud of the education that I was fortunate to receive at the University of Maryland. I feel that it is a good foundation from which to build and grow in the profession."

Constructive comments made by graduates will be considered in future program planning. Several of these comments included:

"Increase the clinical experience with regard to quantity, but not just by raising requirements. There is a need to change the whole system to facilitate more clinical work; such as block assignments and better clinical instruction in the senior year."

"Place less concentration on the one 'right way' to treat a particular situation, and more exposure to different techniques. Provide the opportunity for more instruction in patient management."

As indicated in the questionnaires, some of the graduates appreciated the opportunity to comment on their dental education at the University of Maryland.

Overall evaluation by Program Directors/Associates of our graduates was favorable. The majority of graduates were judged by their supervisors to be within the "average" to "exceptional" range in all clinical and didactic categories, with "good" to "outstanding" ratings on professional attitudes. When asked, "In retrospect, would

this individual be an appropriate candidate for your program or practice?", forty-one (89%) answered "yes," two (4%) answered "no," and three (7%) did not respond.

Several comments provided by the Program Directors/Associates about our graduates included:

"It has been a pleasure to work with Dr. X. He knows his limitations and readily seeks advice and can discuss problems with an open mind. He has performed well as a general practice resident making good progress in expanding and improving his clinical proficiency."

"The type of individual we love to have in our program. He doesn't have to be pushed. He is real steady, a strong person."

"Dr. X possesses good training and attitude toward dentistry, but lacks a sensitive relation to patients at this time (more involved with dentistry than the patient). I feel that he will learn his limitations and become more aware of patients' anxieties."

"Dr. X has been with me since September. He has performed more capably than any associate before in this office. . . . I feel that he undoubtedly has the best background training of any previous associate."

DISCUSSION

The subjective nature of the study allows only for interpretation of individual responses, thereby precluding a generalized sum-

mary of the results. Important and useful information, however, for both the School and its present students was elicited.

The Dental School is presently evaluating many of the suggestions concerning the dental curriculum made by graduates and their Program Directors/Associates. The evaluations of individual programs/experiences by graduates will be useful in counseling

students concerning career/program options following graduation.

The study will be repeated with future graduating classes in an effort to accumulate comparative data for trend analysis.

The Dental School is cognizant of the value of program evaluation of and by its graduates and welcomes written comments.

THE HAIRLESS MOUSE:

A selected literature review

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THE HAIRLESS MOUSE: A selected literature review*

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ABSTRACT

Hairless mice have been reported in the literature for over 150 years. This characteristic was initially regarded to be a pathological condition but was later noted to be an inheritable characteristic. Although hairless mice are of interest both from a genetic and morphologic point of view, their primary use has been restricted to investigations of dermatological nature. The genetics of this and similar characteristics are discussed.

Hairless mice (figure 1) have been reported in the literature for over 150 years. A rare variety of house mouse (*Mus musculus*), naked except for its snout, ears, tail and lower half of legs, was observed by Gordon (1850). Gaskoin (1856) reported the capture of a mouse with a pinkish white integument possessing no hair except on the lips, which was apparently identical to that of a preserved specimen of Clift (recorded in the

Catalogue of Monsters in 1820). Marshall (1887) also observed what appeared to be a totally hairless common house mouse.

Most of the hairless animals described in the literature were a variety of the common house mouse, *Mus musculus* (Gordon, 1850; Gaskoin, 1856; Pocock, 1904; Brooke, 1926; Snell, 1930; Howard, 1940; Packchianian and Rigdon, 1970), although, Sumner (1924) and Egoscue (1962) studied a strain of hairless deer mouse, *Peromyscus maniculatus*. While both of these mutants appear to possess recessive genes for hairlessness, dominant forms also exist (Steinberg and Fraser, 1946; Mann, 1963; Tsuji and Yosida, 1965; Flanagan and Isaacson, 1967) which differ from the recessive animal in the form and manner of hair loss.

Allen (1904) regarded hairlessness to be a pathological condition that might be a heritable characteristic. Brooke (1926), on the

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Figure 1. Photograph of a hairless mouse. Notes the presence of vibrissae (arrow) and the lack of body hair.

other hand, noted that the hairless condition was not pathological and further observed that hairless mice appear normal until 12-15 days of age after which shedding of hair is initiated. These findings were corroborated by other investigators who additionally noted that depilation commenced around the eyes and feet and progressed caudally (Campbell, 1907; Snell, 1930; Crew and Mirskaia, 1931; David, 1932; Howard, 1940; Steinberg and Fraser, 1946; Montagna, Chase and Melargno, 1952; Mann and Straile, 1961; Yun and Montagna, 1961; Hosek, Chlumecky and Mistustova, 1965; Orwin, Chase and Silver, 1967; Chlumecky, 1967; Iversen and Iversen, 1967) with almost complete depilation by 20-21 days of age (David, 1934; Howard, 1940; Mann and Straile, 1961;

Chlumecky, 1967; Spalding and Brooks, 1967; Iversen and Iversen, 1967; Mann, 1971).

David (1932) indicated, and others concur, (Howard, 1940; Mann and Straile, 1961; Mann, 1971) that hairless mice also lose their vibrissae but not at the time of the depilation, a process which is mostly completed by the age of three weeks. Vibrissae, however, became increasingly sparse with age and were eventually replaced by abnormal hair (Howard, 1940; Mann and Straile, 1961; Mann, 1971). Howard (1940) noted that the initial loss of vibrissae occurred at about one to three months of age. Other investigators noted only virtual hairlessness except for the presence of vibrissae and some regionally distributed clumps of

hair (Gordon, 1850; Gaskoin, 1856; Campbell, 1907; Sumner, 1924; Crew and Mirskaia, 1931; Keeler, 1931; Montagna, et al., 1952). David (1932) explained the time differential by showing that hair loss commences at the end of the hair growth cycle, and vibrissae have a longer growth period.

Subsequent to the first loss of hair, a second sparse pelage begins to grow (Snell, 1930; David, 1932; Steinberg and Fraser, 1946; Chase, 1954; Mann and Straile, 1961; Montagna, et al., 1952; Yun and Montagna, 1961; Chlumecky, 1967; Iversen and Iversen, 1967) but it, too, is shed by about the age of forty days (Yun and Montagna, 1961; Mann and Straile, 1961). Other studies report that this second hair loss occurs at a later date, forty-five days (David, 1932), fifty-four days (Spalding and Brooks, 1967), and sixty days (Iversen and Iversen, 1967). Rigdon (1975) noted that, in his mice, hair develops normally, but by the end of the first week it begins to disappear and by the 30th-40th day all hair with the exception of the vibrissae is gone.

The integument of the hairless mouse thickens progressively with age (Crew and Mirskaia, 1931; David, 1932) eventually becoming three times the normal (Keeler, 1931). Crew and Mirskaia (1931) suggested that thickening may facilitate heat conservation and compensates for the lack of hair. David (1932, 1934) and Grüneberg (1947) reported that the increased thickness of the dermis was due to extensive cyst formation, a condition which is also responsible for a whitening, folding and wrinkling of the skin. Folding and wrinkling

of the skin has been observed frequently (Gaskoin, 1856; Pocock, 1904; Allen, 1904; Campbell, 1907; Sumner, 1924; Keeler, 1931; Crew and Mirskaia, 1931; David, 1932, 1934; Howard, 1940; Steinberg and Fraser, 1946). At times it occurs to such an extent that the skin resembles that of a "rhinoceros" and is generally referred to as "rhino" (Gaskoin, 1856; Pocock, 1904; Campbell, 1907; David, 1932; Howard, 1940; Steinberg and Fraser, 1946).

Age-related cyst formation in the skin of the hairless mouse has been noted by many investigators (Crew and Mirskaia, 1931; Keeler, 1931; David, 1932; Grüneberg, 1947; Chase and Montagna, 1952; Montagna, et al., 1952; Yun and Montagna, 1961; Iversen and Iversen, 1967; Meier, Myers and Huebner, 1969; Mann, 1971; Rigdon, 1975). David (1932) suggested that the thickening and wrinkling of the skin was a function of the degree of cyst formation. This condition occurs at about two months of age (Montagna, et al., 1952), that is during the second hair growth phase of thirty to fifty-five days of age (Iversen and Iversen, 1967). Meier, et al. (1969) suggested that a leukemic condition may be implicated in the cutaneous cyst formation in the hairless mouse. Also, with age, the cells of the abnormal hair follicles become stranded and they aggregate to form cysts (Montagna, et al., 1952; Chase and Montagna, 1952; Mann, 1971), of which there may be three types: follicle-end cysts; sebaceous gland cysts and hair follicle cysts (David, 1932). Montagna, et al. (1952) noted that all cysts possessed sebaceous cells nearby, though Mann (1971) stated that

the sebaceous cells were not necessary for their formation.

The skin of the hairless mouse was examined by Crew and Mirskaia (1931), who noted that the corium was considerably thickened and sebaceous glands were degenerated, forming cysts. They additionally noted loss of hair including the follicle and degeneration of the papilla. These data were corroborated by David (1932) who contrasted this type of hair loss with the breaking off of the hair observed in dominant hairlessness.

Failure of the hair club to form properly has been identified as the causative agent in the total loss of hair (David, 1932; Grüneberg, 1947; Chase, 1954; Montagna, et al., 1952; Orwin, et al., 1967). Chase (1954) has traced this abnormality to the connective tissue sheath, a mesodermal component of the follicle. The deposition of collagen in the skin of the hairless mutant is reduced and the anchoring brush fails to form (Orwin, et al., 1967). Hair germs and papillae may produce successional hairs, the second pelage, but these are usually abnormally directed (David, 1932; Montagna, et al., 1952).

The hairless mouse is a difficult animal to raise (Snell, 1930; Crew and Mirskaia, 1931; David, 1932). Though the weight of the hairless mutant is normal at birth (David, 1932), the death rate is particularly high at five weeks of age (Crew and Mirskaia, 1931) and by the eighteenth month of life 72% of these mice contract leukemia (Meier, et al., 1969). Consequently, the earliest encounters with these mutants were brief. For example, the captured adults of Gor-

don (1850) lived only a few weeks, and in the case of those of Gaskoin (1856) two were dead within twenty-four hours while the other two died shortly thereafter. Of the litter from a hairless female obtained by Allen (1904), only one survived past two weeks, Campbell's (1907) specimens lived less than six months (Sumner, 1924) and Snell (1930) and David (1932) noted that the general vigor of these mutants was somewhat less than that of normal mice. It has been noted further that the hairless mutant is very susceptible to death by anesthesia (Crew and Mirskaia, 1931).

Hairlessness in mice is also associated with a defect in the female mammary glands, and is manifested as a lack of mammary tissue including the ducts leading to the nipples (Crew and Mirskaia, 1931). Additionally, the female mutant either does not nurse her young at all or nurses only the first litter but none thereafter (Crew and Mirskaia, 1931). On the other hand some investigators (Howard, 1940; Grüneberg, 1947; Green, 1966) only mention a reduced ability to nurse and the offsprings are usually given to a surrogate mother (Snell, 1930; Crew and Mirskaia, 1931; Howard, 1940).

Hairless mice consume a greater amount of food than normal mice (Crew and Mirskaia, 1931; David, 1932; Kreyberg, Iversen and Iversen, 1965), which has been implicated with an abnormally high metabolic rate (David, 1932; Hosek, et al., 1965; Hosek and Chlumecky, 1967; Tsuji, 1971). Oxygen consumption and the carbon dioxide output of hairless mice were

greater than those of the normal mouse. The heat output is nearly twice that of normal in the four week old group, and the internal temperature of the mutant is lower than that of the normal strains (Hosek and Chlumecky, 1967).

The male hairless mouse has been generally reported to be fertile (Crew, 1927; Crew and Mirskaia, 1931; Keeler, 1931; David, 1932; Howard, 1940; Steinberg and Fraser, 1946; Hosek, et al., 1965) and the greatest degree of fertility in the male occurred following sexual maturity (one to two months of age) and declined sharply with age (David, 1932).

Much of the earlier literature indicated that hairless females were either totally sterile or usually sterile (Campbell, 1907; Sumner, 1924; Crew, 1927; Snell, 1930; Keeler, 1931). Allen (1904) reported, however, on one hairless female that gave birth to a hairless offspring. Recently, Egoscue (1962) reported that hairless females are almost uniformly sterile, but Crew (1927) noted that fertility is induced in normally sterile females by raising the ambient temperature to 30° C or higher, while partial or near normal fertility has been reported in hairless females (Crew and Mirskaia, 1931; Howard, 1940; Steinberg and Fraser, 1946; Hosek, et al., 1965). Crew and Mirskaia (1931) noted that 50% of their hairless females failed to breed even though in estrus, and that a normal haired male would not mate with a receptive hairless female, even though she subsequently accepted a hairless male.

In breeding for hairlessness the problem of sterility in the female

hairless mouse may be circumvented, in part, by crossing a hairless male with a normal haired female and either back-crossing the F₁ female or by mating her to another hairless male (Sumner, 1924; Keeler, 1931; Crew and Mirskaia, 1931; Montagna, et al., 1952; Mann and Straile, 1961; Spalding and Brooks, 1967; Tsuji, et al., 1969; Packchianian and Rigdon, 1970; Argyris and Argyris, 1970; Mann, 1971).

Crew and Mirskaia (1931) believe that the increased viability of the hairless animal might also be due to the dilution of the deleterious characteristics by breeding the mutant with a normal wild type female, which consequently results in a more vigorous animal possessing a more balanced gene pool.

Hairless mice develop elongated, curled toe nails (Sumner, 1924; Crew and Mirskaia, 1931; David, 1932; Howard, 1940; Steinberg and Fraser, 1946; Grüneberg, 1947; Egoscue, 1962; Chlumecky, 1967) beginning at about five weeks of age (Crew and Mirskaia, 1931; Howard, 1940), but Steinberg and Fraser (1946) noticed that the abnormal claws were less extreme in the smooth skinned hairless mice than in the rhino hairless mice.

The recessive hairless mouse (*Mus musculus*) has been described both as a smooth skinned animal as well as a "rhinoceros" animal. Snell (1930) pointed out the possibility that his smooth skinned hairless mouse exhibited a type of hairlessness different from that of the wrinkled specimens described in the earlier literature by Gaskoin (1856), Poock (1904), Allen (1904) and

Campbell (1907). David (1932) also observed that some hairless mice were relatively smooth skinned, while others were quite wrinkled. Howard (1940), using the hairless and rhino hairless mutants, determined that the rhino characteristic was recessive to the hairless characteristic; on the other hand Steinberg and Fraser (1946), suggested that the rhino characteristic is not completely recessive to hairlessness. Both animals are homozygous recessive and have been assigned genetic symbols of hr/hr and hr^{rh}/hr^{rh} for hairless and rhino, respectively.

Possible confusion that may exist when mutant animals appear similar but differ genetically and morphologically (Mann, 1971). Such confusion is frequently encountered in the literature regarding hairless mice since many investigators did not note the origin or genotype of the homozygous recessive hairless mouse they used. In the earlier literature (Gordon, 1850; Gaskoin, 1856; Pocock, 1904) even the dominant or recessive nature of hairlessness could not be determined because of the brevity and vagueness of the descriptions. The hairless mouse utilized by Crew and Mirskaia (1931) and David (1932) were obtained from Brooke's (1926) stock, while Howard (1940) and Mann (1971) obtained his hairless animals from the F_5 generation of a stock which had its origin from a cross between two highly inbred strains. Spalding and Brooks (1967) obtained a homozygous recessive hairless mouse from a single inbred strain, while Chlumecky (1967) obtained a new strain of hairless mice from a non-inbred strain. Most other investigators

obtained their mutants by inbreeding various strains of heterozygous hairless mice (Montagna, et al., 1952; Mann and Straile, 1961; Yun and Montagna, 1961; Orwin, et al., 1967; Tsuji, et al., 1969; Tsuji, 1971).

Although hairless mice are of interest both from a genetic and morphologic point of view, their primary use has been restricted to investigations of dermatologic nature (Tsuji, Sugai and Saito, 1969; Gates, Arundell and Karasek, 1969; Laerum, 1973; Constantine, Francis and Mason, 1971; Orris, 1969; Rigdon, 1975; Kapral and Miller, 1972; Iversen, Iversen and Bjerknes, 1968). Recently, however, they have been utilized in immunological studies involving the thymus (Prabhakaran, Harris and Kirchheimer, 1975). As yet, no literature has appeared relative to the development of its dental tissues, though the adult has been described as possessing "normal" (Sumner, 1924; David, 1932; Howard, 1940) as well as "abnormal" dentition (Crew and Mirskaia, 1931). Since hairlessness appears to be related either to abnormal development of an ectodermally derived tissue, or its interaction with the subjacent mesenchyme, a study of odontogenesis in the hairless mouse, which involves similar developmental mechanisms, would be of great interest to oral biologists. Such an investigation is presently underway in our laboratory, and will be the subject of future communications.

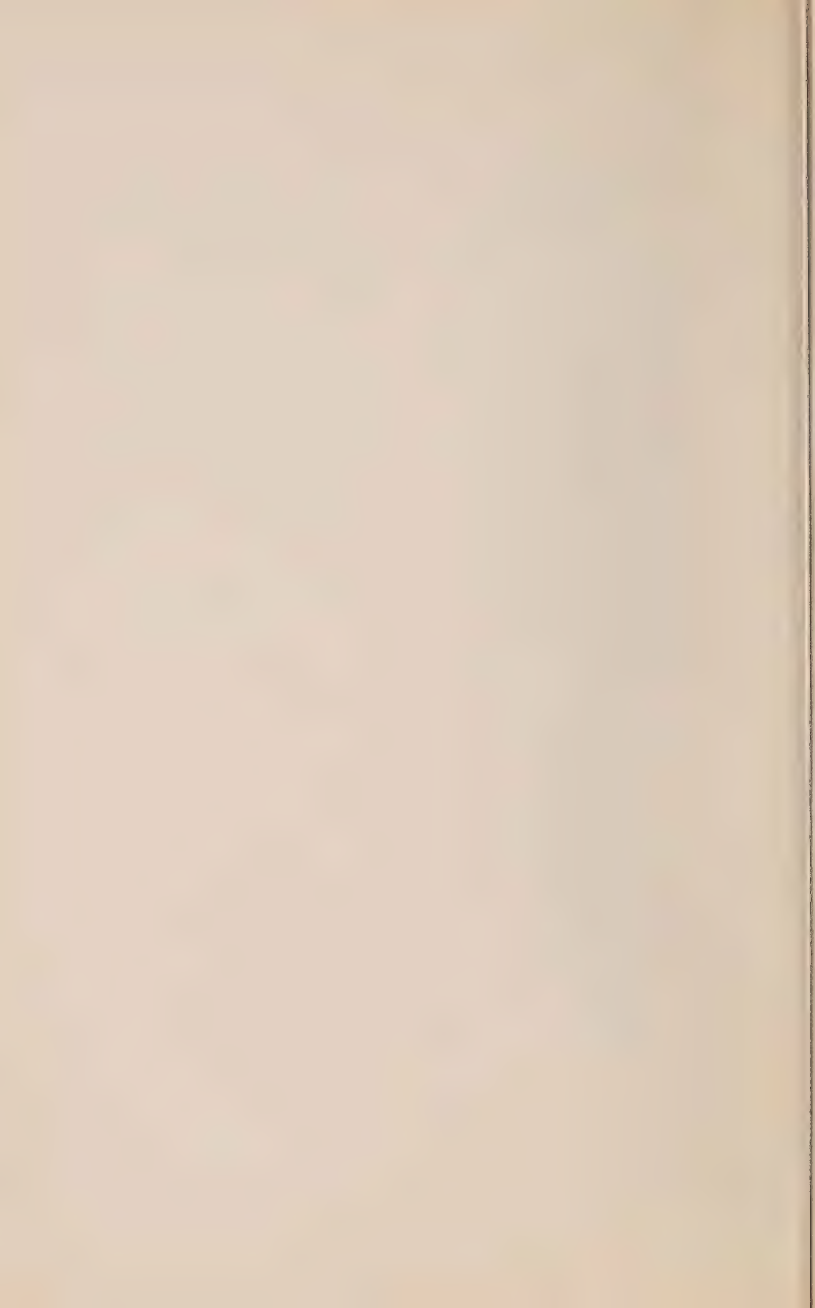
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Indications of the Sanitation Level in a Dental Clinic

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PREFACE

The following study was conducted during clinic hours and under actual working conditions at the Dental Clinic, Baltimore College of Dental Surgery, University of Maryland. In order to maintain the existent working situation in the clinic, little attempt was made to control variables which consequently were introduced into the study. The authors were more concerned with analyzing a situation in its existent form. In so doing, we appreciate the necessary cooperation of the many students, faculty and patients who were called upon without prior notification to participate. While some potential problems have been identified, the conscientiousness and detail necessary for good dental operatory procedures were observed to be characteristic for the majority of the student population in the dental clinic. A special tribute belongs to the administrators, faculty and students who have taken appropriate action to resolve the problems identified here.

SUMMARY

Some indication as to the sanitation level of the dental clinic at the University of Maryland School of Dentistry has been determined.

Areas in the student operatories including instruments readied for patient use were examined for the presence of microorganisms. The areas sampled included the floor, walls, counter tops, headrest, headrest cover, light handle, chair control buttons, sleeve cuffs of clinic jackets, paper towels, drinking cups, hands and instruments. Appropriate flat areas were sampled using rodac surface sampling plates containing blood agar. Other areas were sampled by swabbing and placing the swab in a diluent or directly placing the object into ten ml of sterile saline solution.

The results indicate that the areas in need of immediate attention include the hands, clinic jacket cuffs, the light handle, chair control buttons, floor and the instruments.

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INTRODUCTION

A microbiological survey has been conducted in the general patient clinic area at the School of Dentistry, University of Maryland. The clinic examined has a student population of two hundred forty (240). Each student has his own individual operatory in a partitioned cubicle which has an approximate area of fifty-five (55) square feet. Thirty-two (32) cubicles are contained in a single area designated as a module. The entire clinic area consists of eight (8) modules.

The primary objective of the surveillance was to get some indication as to the sanitation or cleanliness level in the clinic area based on microbial population.

The primary stimulus for such an undertaking was recognition of the necessity to know and understand more about the general sanitation under which dental procedures were being conducted in the dental clinic and to encourage appropriate action on the part of the clinic faculty and students.

A second stimulus was the result of a preliminary study done last summer which indicated that several areas in the clinic may be potentially hazardous as a possible source of microbial contamination and/or infection.

Consequently, this study was initiated in October 1974, and extended through February 1975. As a result of the previous preliminary report, several modifications were made in the surveillance procedures. More specifically, the number of subjects and and subject areas tested, which included

the individual student cubicles, was increased to include thirteen (13) percent of the clinic student population. Additionally, several of the procedures used to sample objects were modified to produce a survey system with greater sensitivity.

The specific areas examined were selected, based again on the results of the preliminary study. This study showed that major areas of concern should include the operator's instruments, hands, clinic jacket cuffs, the light handles, chair control buttons, floor, walls and counter top areas. Sampling of the air in the module areas was also done.

MATERIALS AND METHODS

Two subjects were examined on each sampling day. Each subject was surveyed twice during the day: in the morning prior to patient contact and again in the afternoon after having seen at least a single patient. The general protocol of the survey follows.

A survey kit consisting of the required media, swabs and saline solutions, was taken to the clinic between 8:30 a.m. and 8:45 a.m. One of the several subjects who had been randomly chosen several days prior to the actual sampling day was selected. Each operator was approached with the statement that he had been selected on a random basis to participate in a microbiological survey of the dental clinic. The operator was then instructed to continue to make preparations for his patient appointment in a routine and normal manner. Meanwhile, the technicians began sampling the floor and wall areas. The technicians then



Figure 1. Culturing of a Flat Surface Area (Counter top) using the Rodac surface sampling plate containing blood agar.

permitted the operator to complete preparations for the patient's visit. After all preparations were completed, the team of technicians collected samples of the instruments and areas, as indicated below:

A single rodac plate containing blood agar was used to sample the following areas : (1) floor, (2) right side of rear cubicle wall, (3) left side of rear cubicle wall, (4) counter top—right side of cubicle, (5) counter top—left side of cubicle, (6) light handle, (7) right cuff of clinic jacket, (8) left cuff of clinic jacket, (9) the bracket tray, (10) headrest cover, (11) headrest, (12) paper towel for drying

hands, and (13) drinking cups. (Figure 1).

The mirror, scaler, probe and two curettes were sampled collectively by immersing and gently agitating the ends in ten ml of sterile saline contained in a fifty ml sterile beaker which was kept covered with aluminum foil. (Figure 2) Four burrs were separately placed in an identically filled beaker and gently agitated for approximately fifteen seconds.

The control buttons on the dental chair were sampled by swabbing with a sterile cotton swab which had been wetted with sterile saline immediately prior to swab-



Figure 2. Collective culturing of several dental instruments by immersion and agitation in sterile saline. The saline was then serially diluted and plated in duplicate onto blood agar.

bing. All control buttons on only one side of the chair were so sampled. The side sampled was based on whether the student operated on the right or left side of the chair. After sampling, the swabs were placed in a tube containing ten ml of sterile saline.

The water injector and the handpiece were sampled by immersing the tips in a tube containing ten ml of sterile saline.

The air around the cubicle area was sampled by removing the lids of four petri plates containing blood agar for a period of ten minutes.

Each of the operator's hands was immersed completely and agitated in 100 ml of sterile saline contained in a plastic bag. (Figure 3). This was done immediately prior to the operator examining the oral cavity of the patient and in most cases, after washing.

An effort was made to have all samples taken before the patient arrived and was seated in the dental chair, with the exception of the hand samples. Once the patient was seated the technicians stood by until the operator washed his hands. Immediately after washing and drying, the hands were sampled. This completed the morning

portion of the survey. The subject was not told that he would be asked to participate again in the afternoon. However, near the latter part of the total study, some subjects had become aware of the protocol to be used.

The team of technicians returned to the clinic in the afternoon to conduct the second phase of the survey which was done in the exact same manner as described for the morning.

Following sample collections in the clinic, the technicians returned to the Central Laboratory Services' Microbiology Laboratory. The rodac agar plates (used to sample

flat areas) and the blood agar plates (used in the air sampling procedure) only required incubation at 37°C. The samples of instruments, chair control buttons, water injectors and hands were taken in saline solution and thus required dilution followed by the plating of each dilution onto blood agar plates. All samples requiring dilutions, except the hands, were diluted 1:100 in two steps, each step being a 1:10 dilution. The hands were diluted 1:1000 in three steps, each step being a 1:10 dilution. Sterile saline was used as the diluent in all cases. Each dilution (1:10 or 10^{-1} , 1:100 or 10^{-2} , and for the hands, 1:1000 or 10^{-3}) was spread plated onto two blood agar

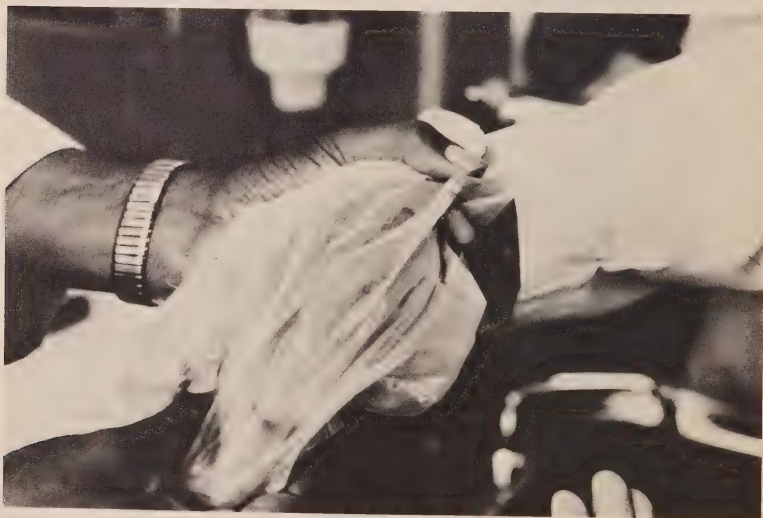


Figure 3. Culturing of the operator's hand immediately prior to patient contact—was done by immersing each hand into a plastic bag containing 100 ml of saline solution. The solution was gently agitated about the hands by squeezing the bag.

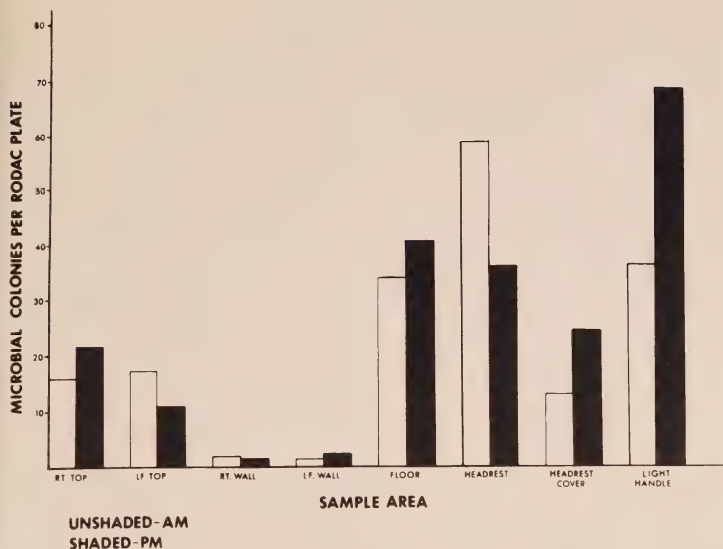


Figure 4a. Shown above are the average counts from the various flat surface areas tested.

plates. The blood agar plates were properly labelled and incubated at 37°C.

All plates were incubated for 24 hours. They were then inspected, and those in which there was no overgrowth were re-incubated for an additional 24-hour period. Following this, all plates were removed from the incubator. All microbial colonies on each plate were counted, and the counts were recorded.

In a few instances when bacterial colonies appearing on the plates seemed to be characteristic of a pathogen, biochemical tests were done to confirm the identity of the

organism. Available resources did not permit this to be done in every case in which a pathogen was suspected.

DATA

The results of the microbial counts from the rodac plates for each of the subjects and subject areas tested appear in Table I. The figures presented represent the total and average number of microbial colonies appearing on the rodac plates for each area tested. According to the manufacturer's specifications, the surface area tested by each rodac plate is four square inches. The averages were used to plot the vertical bar graphs in Figures 4 and 5.

The counts of the instruments and other areas which were sampled in the saline solution are presented as the number of microorganisms per milliliter of solution in Table II. The figures in this table represent an average of two plate counts since each dilution made of the saline suspensions was plated in duplicate. Again, averages from the thirty (30) subjects were obtained for each of the areas tested. The averages appear in Table II and are the basis for the bar graph presented in Figure 5. Figure 5 was plotted on semi-logarithmic graph paper in order to accommodate the high microbial counts

obtained from the hands. The counts from the other instruments sampled in saline solution were also plotted on the same type of graph paper in order to maintain consistency. The average figures which were calculated in the tables and from which the bar graphs were prepared were again based on the count from each subject from which a countable sample specimen was obtained.

The averages for the headrest cover, the headrest, the paper towels, the drinking cups and the cuspidor were calculated, using considerably less than thirty (30)

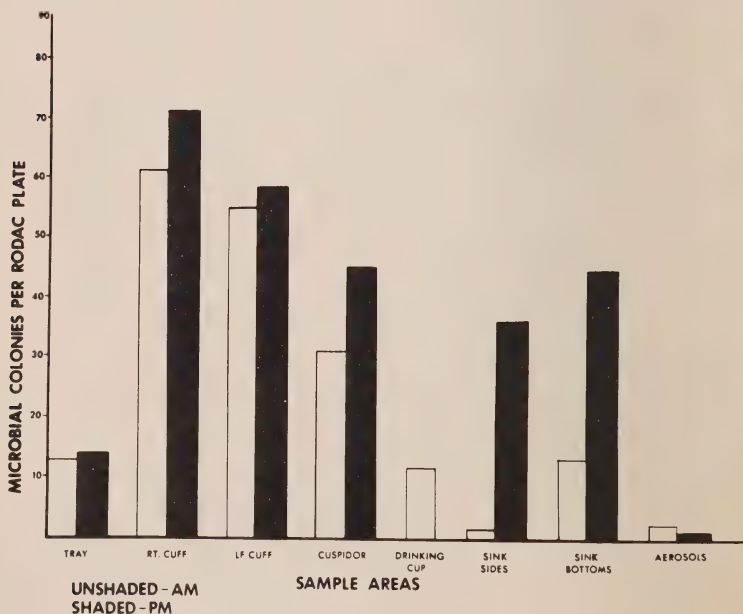


Figure 4b. Shown above are the average counts from the various flat surface areas tested.

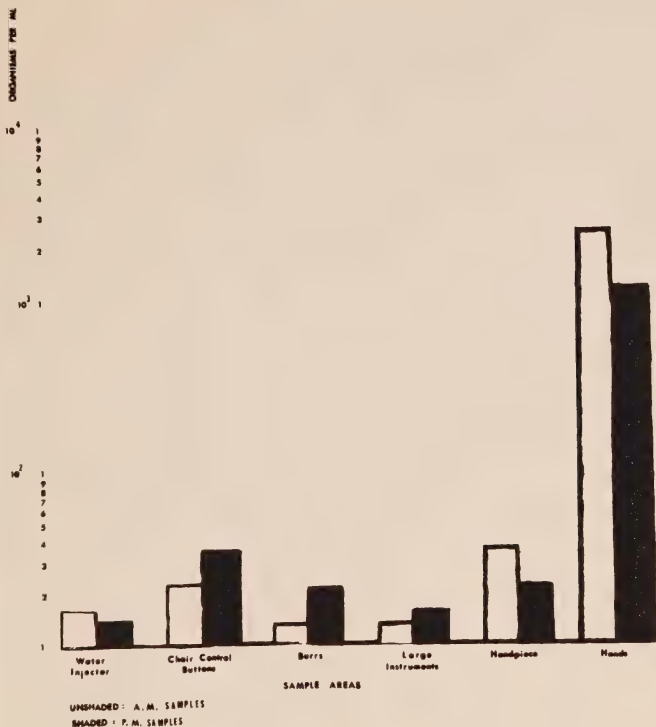


Figure 5. Average counts from morning and afternoon culturing of student instruments and hands. Plots were made on semi-logarithmic graph paper.

samples because these items were not sampled continuously in the survey. It was determined after several samples, for example, that the counts obtained from the paper towel and the drink cup were negligible. These samplings were then discontinued. The cuspidor was seldom used by the operator and was sampled infrequently.

Since the counts from the paper towels were insignificant, figures representing an average count were not included in the bar graph. The average figure for paper towels in the morning was 0.4 and in the afternoon, 0.0.

Only one bar is presented for the drinking cup. The morning

TABLE I
Microbial Plate Counts From Flat Surface Samples

SAMPLE AREAS	TOTAL MICROBIAL COUNT		AVERAGE MICROBIAL COUNT		NO. OF COUNTABLE SAMPLES	
	A. M.	P. M.	A. M.	P. M.	A. M.	P. M.
Right Counter top..	445	660	14.8	22	30	30
Left Counter top ...	484	314	16.1	10.4	30	30
Right Wall.....	50	35	1.7	1.2	29	30
Left Wall.....	42	44	1.4	1.6	29	29
Headrest.....	1,236	610	61.8	35.8	20	17
Headrest cover.....	332	525	12.8	25	26	21
Tray.....	380	415	12.7	13.8	30	30
Floor.....	965	1,208	32.1	40.2	30	30
Light Handle.....	1,051	2,058	35	68.6	30	30
Right Cuff.....	1,762	2,108	60.8	72.68	29	29
Left Cuff.....	1,586	1,709	54.7	58.9	29	29
Sink Sides.....	35	1,102	1.1	36.7	30	30
Sink Bottoms.....	369	1,355	12.3	46.16	30	30
Cuspidor.....	100	135	33.3	45	3	3
Paper towel.....	4	*	.36		11	
Drinking cup.....	113	*	11.3		10	
Aerosols.....	69	49	2.3	1.6	30	29

* No samples taken.

figure is 0.0, hence no bar is illustrated.

The counts on the aerosol plates are given as counts per plate. The petri plates used were size 100 x 15mm. Averages for the aerosol plates were obtained and are seen in Table I; this average is also indicated in Figure 4b.

There were instances where too many colonies appeared on a plate to obtain an accurate count. In other cases, there appeared a countable number of colonies on the plate; but because the growth of one or more colonies spread over the surface of the agar masking other colonies present, it was impossible to obtain an accurate count. In these instances, no plate count was recorded. Where no numerical count or other designation appears in the table a sample was not taken.

DISCUSSION OF RESULTS

The results of this study give a clear indication as to the sanitation level of most of the areas and/or instruments surveyed. However, there are some cases in which explanations offered as to the obtained results must be speculative.

Since this study was concerned with obtaining an index of the cleanliness level, both in the morning prior to any operative procedures and again in the afternoon following at least a single patient visit, one would expect the microbial population in the later analysis to be increased (an increased microbial population is interpreted as a decreased sanitation level). While this expectation bore out in averages of the counts of thirteen of the areas routinely tested, it did

not on seven of the others (Figures 4 and 5). The seven included: the headrest, the left wall, the aerosols, the water injector, the handpiece, the hands and the left counter top. The difference in the results on the left wall, the aerosol and the water injector was probably not sufficiently large enough to be statistically significant. The reasons for the noted decrease in the afternoon microbial count following patient contact and the performance of operative procedures can again only be speculative. Surmising then, several factors could have influenced this type of result:

- (1) The student immediately initiated steps to improve the cleanliness level of his operative area (including instruments).
- (2) The procedures performed in the morning provided accidental cleaning by a reduction of the microbial population of some areas.
- (3) A new and different object was used for testing in the afternoon.

It appears that perhaps factor (1) above could possibly apply to results obtained with the handpiece, the hands and the left side counter top. Certainly the handpiece, if used in the morning, would contain an increased microbial population if it were not disinfected in some manner. It was observed that the majority of the operators were especially conscious of the necessity of at least a minimum cleaning of the tip of the handpiece. This was accomplished usually by wiping with alcohol-wetted (as opposed to soaked) gauze squares. Also, the students

TABLE II
Counts per millimeter of Microbial Colonies Recovered From Dental Instruments and Hands

SAMPLE #	WATER INJECTOR		BUTTONS		BURRS		LARGE INSTRUMENTS		HANDPIECE		HANDS	
	COUNTS/ML		COUNTS/ML		COUNTS/ML		COUNTS/ML		COUNTS/ML		COUNTS/ML	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
1	5	5	5	0	0	0	0	0	0	0	210	320
2	0	0	15	0	0	0	0	0	0	0	600	3,600
3	5	33	15	60	0	0	0	0	405	0	5,100	780
4	0	0	0	15	20	460	0	0	10	0	6,770	2,450
5	35	0	45	10	0	0	0	0	0	0	4,375	730
6	5	25	0	5	0	0	0	15	0	0	440	435
7	0	5	270	0	0	0	10	0	0	0	785	145
8	0	15	0	10	0	0	0	0	135	0	60	100
9	0	0	0	95	0	0	0	0	0	0	4,550	545
10	0	5	0	5	0	0	65	0	0	0	35	
11	20	0	5	5	0	0	0	5	0	15	250	155
12	0	5	5	0	0	0	0	0	0	85	60	
13	10	0	0	0	0	0	0	0	30	10	170	395
14	0	0	10	5	0	0	0	0	0	0	470	415
15	0	10	50	0	0	0	5	0	0	0	2,100	1,050
16	110	45	100	0	0	0	5	0	0	45	2,135	725

Continued

TABLE II—Continued

SAMPLE #	WATER INJECTOR		BUTTONS		BURRS		LARGE INSTRUMENTS		HANDPIECE		HANDS	
	COUNTS/ML		COUNTS/ML		COUNTS/ML		COUNTS/ML		COUNTS/ML		COUNTS/ML	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
17	0	0	5	175	0	0	0	5	0	0	495	410
18	30	0	5	0	5	140	0	10	0	0	240	560
19	70	0	130	5	0	0	0	0	5	0	80	165
20	0	10	10	35	0	0	0	0	5	0	220	530
21	0	0	20	0	0	0	0	15	0	0	345	225
22	0	0	15	20	0	0	0	0	115	485	375	570
23	0	0	0	5	25	0	90	12	0	0	1,365	2,475
24	0	0	10	10	0	0	5	0	0	0	105	745
25	65	40	0	615	0	0	0	0	0	0	2,190	270
26	0	5	0	5	0	5	0	270	0	0	2,000	1,125
27	0	0	5	0	125	0	5	0	15	0	1,970	1,045
28	0	0	0	0	5	0	0	0	0	0	35	50
29	20	0	0	0	0	0	0	0	0	0	515	9,500
30	20	0	0	0	0	0	0	0	365	5	500	2,040
TOTAL Microbes/ml	395	203	720	1,070	180	605	185	332	1,085	645	38,545	31,555
AVERAGE Microbes/ml	13.2	6.8	24	35.7	6	20.2	6.2	11.1	36.2	21.5	1284.8	1051.8

seemed more conscious of doing this in the afternoon, since it was more obvious that this instrument had just been used in a patient's mouth; whereas, in the morning this may not have been as obvious, since the last patient was 'yesterday.'

Factor (3) is also possible with the handpiece wherein a different handpiece was chosen for use in the afternoon.

The hands could produce such results because of factor (1) also, although another element could be the residual antimicrobial effect of the handwashing compound used. Some soaps are known to produce this effect with consistent use. Operators may have washed their hands several times before the afternoon sampling was done, resulting in an accumulation of residue.

The reduced headrest count in the afternoon may possibly be due to factor (2). By changing the headrest cover, it is feasible that many microbes were removed with the cover.

As stated previously, the trend of the data on the other areas collected was as one would predict; based on the preliminary survey conducted last summer.

First, to review the results of the surface areas which were tested using the rodac surface sampling plates (Table I and Figure 1), all of the results from these tests can be compared since the surface area tested was the same in all cases. In this regard then, one can see that the "cleanliness level" of the right jacket cuff is the lowest or 'worst' of all the other areas

tested, including the floor. When questioned about the frequency in change of clinic jackets, most operators indicated a weekly change was routine; others changed every two weeks. Few indicated it was routine to change more than once weekly. That the cuffs would have a low level of cleanliness is predictable, since the cuff is perhaps a collector of much of the aerosol generated during the use of high speed handpieces and other dental procedures. Such operations as scaling also cause materials to be projected out of the mouth much of which may also be deposited on the jacket cuff.

The area with the second highest count was the light handle. As expected, the afternoon count was much higher than the morning count. In fact, the afternoon average of almost seventy microbial colonies per rodac plate was nearly twice that of the average from the morning results. This undoubtedly resulted from the high amount of use and hand contact with which the light handle was subjected.

The low cleanliness level of the headrest was probably due to the fact that it is not looked upon as being a "critical area" to be sanitized or disinfected as some other areas. Consequently, the headrest does not receive proper attention. Also, many operators perhaps feel that the use of the headrest cover gives sufficient protection from the possibility of contamination or infection.

The cuspidor, as indicated in the preliminary survey, had a high microbial count and hence a low cleanliness level. It was found that

very few of the operators used the cuspidor during the course of this survey. With the low cleanliness level indicated on the cuspidors examined, it may be advantageous from a sanitation point of view to forego use of this apparatus unless proper procedures are adopted and used to improve its cleanliness level.

Examination of the sinks in the modules was somewhat revealing in regard to improper procedures. The sink sides and sink bottoms showed the highest increase of all in microbial counts from morning to afternoon. The sink bottoms showed an almost four-fold increase. The increase for the sides was higher, being approximately thirty six-fold. This result was not surprising, since the sinks were observed to be used not only for washing of the hands, but for expectorations (mostly by operators), washing of patient's denture models in between fittings, etc. The expectorations were seen to occur as a result of rinsing after toothbrushing as well as from coughs. Often times, expectorating after rinsing was done in such a fashion as to produce splattering. The rather high level of cleanliness of the sinks in the morning must be attributed to the efforts of the housekeeping force.

The floor results have been unexpectedly overshadowed by the data from some of the other areas. The results of the floor, while higher than acceptable as indicated here, must be particularly interpreted cautiously.

The other areas examined with the surface contact plates appeared to be within an acceptable range

even though they may have been in the higher portion of this range.

The resulting data obtained from all of the surface areas sampled with rodac plates is subject to qualification before being interpreted. Such qualification must consider the sampling protocol used and the sampling protocol required for the unquestionable type of interpretations one may be tempted to make based on this study. Pryor and McDuff (1971) have listed the criteria for conducting or implementing a good microbial surveillance system. These include use of a large number of rodac plates for each area tested [a floor which measures 3' x 5' would require a minimum of ten rodac plates; a table top with an area of four square feet (2' x 2') would require a minimum of five rodac plates; sinks require a minimum of three such plates]. The authors further state that "ideally the number of samples taken should be multiplied by three, four or more over what is recommended here." Admittedly, this is unrealistic, except for the largest institutions. A surveillance program which attempts to measure the total surface area of an institution will require thousands of rodac plates and would be impractical except for specific research projects. Pryor and McDuff also indicated that, especially for floor areas, sampling must take place immediately after cleaning and before recontamination has taken place (such as someone stepping on the floor area tested). Further, they suggested that sampling of the same areas must be done routinely and periodically (once a month for some areas) in order to

draw definitive type conclusions from the data obtained.

In view of the above information, the data from this study should be interpreted, being mindful of the objectives and the protocol. The fact that the data obtained in this study is based on an average of the counts from thirty (30) subjects (in most cases) makes it useful for the stated purposes.

In drawing generalized conclusions and in searching for indications, again Pryor and McDuff are referred to, since not enough work has been done to establish a reference for the dental clinic examined. According to these investigators, a rodac plate count of 0-5 is considered good for floors in operating rooms and obstetric wards. Counts of 6-15 are considered fair and above 16 are poor. The same applies for table tops in any area. Floor guidelines for patient rooms are given as: 0-25 = good; 26-50 = fair; and above 51 = poor. For sinks: 0-15 = good, while above 26 = poor.

Keeping in mind that the protocol used in this study was modified for a different objective, indications are that our floors, sinks and counter tops are in need of corrective action which the above authors suggest should start "immediately where consistently high counts occur." Even though no guidelines were available for light handles, jacket cuffs, etc. and since these areas produced higher counts than the floor and sinks, they certainly would be considered far from acceptable. For the purposes intended in this study, any rodac plate counts higher than twenty-five, for any of the areas tested,

would indicate a need for corrective action; and in most cases, can be judged as being in poor condition as far as sanitation is concerned.

The results of the instruments sampled by immersing in sterile saline solution will now be discussed. The large instruments (mirrors, scalers, curettes, etc.) had supposedly been sterilized prior to use. The counts obtained then probably resulted from the practice of removing sterilized instruments from the sterile packing and storing them openly in the glass instrument tray in the operators' cabinet drawers. Preliminary studies showed that although these trays were generally dusty in appearance, the microbial count obtained from them were lower than expected. In other cases, the sterile package would be opened to remove some instruments, and would not then be properly resealed. Still another opportunity for contamination occurred when the instruments were placed on the bracket tray for long periods of time prior to patient arrival. This was sometimes due to patients being tardy for appointments. For the periodontal work, most operators kept the instruments covered with a non-sterile but "sanitized" napkin. Nevertheless, the counts obtained from these instruments are significant, when one considers that procedures were carried out to sterilize them.

In many cases, the burrs were not cleaned in a very efficient manner prior to use. When cleaned, they were wiped with an alcohol-wetted gauze square. This accomplishes superficial cleaning at best, since burrs have crevices. Usually, only the diamond burrs are sterilized by

autoclaving. At least one student was seen immersing and leaving his burrs in alcohol for soaking, prior to use. This is more beneficial than wiping. While the morning count of the burrs may be reasonable since they are not autoclaved, procedures must be initiated to reduce the higher counts obtained in the afternoon.

The handpiece is also normally cleaned by wiping with alcohol-wetted gauze. However, since the handpiece tip is smooth and is made of stainless steel, it is possible to clean it rather efficiently in this manner, contrary to the results obtained. To supplement the procedure used, perhaps the sanitation level of the handpiece tip can be increased by soaking it in alcohol followed by a thorough wiping with alcohol-wetted gauze or by using a more efficient disinfectant (one that is bacteriocidal, sporocidal and fungicidal).

The microbial counts obtained from the water injector tip were low compared to what was predicted by the technicians. This was because of a central crevice located underneath the tip of the water injector, which usually appeared to be "cruddy." However, the action of the water injector may be self-cleaning, preventing a build-up of microorganisms.

The chair control buttons were second only to the hands and the handpiece in this category of sampling technique. This, of course, was expected based on the existent intercontact between the hands and the light handle. Also, the control buttons, like the headrest, was probably not viewed as a critical area to be given attention regarding disinfection or sanitation.

As in the preliminary survey, the hands were the implicated villains when it came to potentially hazardous sources in the clinic area. Not only did the hands produce consistently high counts, but the chances of the hands being a primary focal point of infection or contamination were greater due to direct contact involved with the patient, the instruments and other areas in the cubicle area. Furthermore, more suspected pathogens were seen in the microbes recovered from the hands than any other area. In two instances in which the suspected pathogens were biochemically tested, they turned out to be coagulase-positive *staphylococci* and a *klebsiella* species.

The high counts from the hands could be a result of a improper hand washing procedures or an inefficient handwashing compound. The suggestion is made that the handwashing procedure is definitely deficient. It is usually too brief and too mild (no hand brushes are used to clean beneath fingernails, etc.) to be effective. The soap used cannot be implicated at this point since more specific tests are necessary.

CONCLUSION

This study has demonstrated the areas in the dental clinic at the School of Dentistry, University of Maryland which have a low sanitation level and are in need of corrective action and further study. The study does not, nor was it intended to give unquestionable hard conclusions. Generalized conclusions concerning the most abused areas follow, together with suggestions of corrective action.

The clinic jacket cuffs are definitely considered to be in the potentially hazardous category. They are not only unclean or unsanitary from a microbial basis, but generally, they appear to be lacking in cleanliness. There are several possible avenues of corrective action which may be considered. These would be the use of short-sleeved jackets accompanied by an improved conscientious handwashing procedure. The former, without the latter, may not produce any additional advantages; and a daily change of clinic jackets accompanied by a systematic laundering procedure would be necessary.

The handpiece, chair control buttons, sinks, light handles, headrest covers, hands, burrs and instruments are all in need of immediate corrective action. None of these areas require more than a minor change in the daily preparatory procedures of the clinic operators. As stated by Pryor and McDuff, "the primary value of the surveillance is to encourage extra care on the part of the personnel."

TABLES

Table I—Rodac plate counts for the various surface areas tested. These counts were obtained after 48 hours of incubation at 37°C.

Table II—Counts per ml obtained from the saline solution in which the instruments, chair control buttons and hands were sampled, are presented with totals and averages.

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Management of Dental Patients with Basal Ganglia Disorders

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SUMMARY

A review of the basal ganglia disorders and their pharmacologic treatment is presented in an attempt to point out possible problems of drug interactions or patient management which the dental clinician might encounter in treating patients with these neurological disorders.

INTRODUCTION

A number of neurological disorders are caused by dysfunctions of the major forebrain gray masses generally referred to as the basal ganglia. Anatomically, these areas include the striatum (caudate nucleus and putamen) and the globus pallidus. The resultant diseases include various forms of Parkinson's disease, Huntington's chorea, Gilles de la Tourette's disease and various athetoid and dyskinetic disorders.

In an attempt to restore the balance between the neuro-transmitters which regulate motor control

in the basal ganglia, potent centrally active adrenergic or cholinergic agonists or antagonists are often prescribed. These neurological disorders, their pharmacologic treatments and possible drug interactions will be described.

CLINICAL DISORDERS

Parkinson's Disease — The disease is characterized by a to and fro tremor of the head (3-7 cycles per second) a 'pill rolling' tremor of the hand and fingers, a cog wheel rigidity of the limbs involving both flexors and extensors and by an akinesia (immobility) or bradykinesia (a slowness of movements). There may also be speech impairment, dysphagia, sialorrhea, a sluggish swallowing reflex, seborrhea, orthostatic hypotension and urinary incontinence. For nearly one-hundred years, Parkinson's disease was treated with antimuscarinic (atropine-like) drugs or sympathomimetic amine drugs such as amphetamine and ephedrine. In 1960 Ehringer and Horny-

kiewicz observed that the basal ganglia and the substantia nigra of autopsied Parkinson's patients had reduced concentrations of the central neurotransmitter dopamine, suggesting a possible etiology for the disease. It was subsequently shown that the akinesia and rigidity in patients with Parkinson's disease responded to treatment with *l*-3,4-dihydroxyphenylalanine (L-DOPA)*, the immediate amino acid precursor of dopamine (Birkmayer and Hornykiewicz 1961, 1962; Barbeau, Sourkes and Murphy 1962, and others). It was also observed that drugs which blocked the action of dopamine (such as the phenothiazine tranquilizers) or depleted it (such as reserpine) often produced a syndrome which resembled Parkinson's disease (Carlsson, 1959). Also, since atropine, a blocker of central cholinergic activity, blocked the symptoms of drug-induced parkinson-

ism and relieved the symptoms of Parkinson's disease, it was postulated that Parkinson's disease was caused by a reduction of the inhibitory activity of dopamine on neurons of the basal ganglia resulting in a predominance of cholinergic activity. The disease is currently treated by increasing brain dopamine concentrations *via* administration of its precursor, L-DOPA, or by decreasing cholinergic influence by using a centrally acting anticholinergic drug such as atropine. Either treatment tends to restore the normal balance between dopamine and acetylcholine in the basal ganglia.

Huntington's Chorea—This disorder is manifested clinically by a progressive dementia and chorea (irregular, involuntary action of muscles of the extremities and the face), and pathologically by a loss of small neurons in the caudate and putamen (McMenemy, 1958; Bruyn, 1968). If the cerebral cor-

* See Table I for trade names.

TABLE 1

Drug	Mechanism	Use
L-DOPA (Bendopa, Dopar, Larodopa)	Precursor to Dopamine	Parkinsonism
Amantadine (Symmetrel)	Release dopamine	Parkinsonism
trihexylphenidyl (Artane, Tremin, Piparol)	Anticholinergic	Parkinsonism
ethopropazine (Parsidol)	Anticholinergic	Parkinsonism
benztropine mesylate (Cogentin)	Anticholinergic	Parkinsonism
procyclidine (Kemadrin)	Anticholinergic	Parkinsonism
biperiden (Akineton)	Anticholinergic	Parkinsonism
orphenadrine (Disipal, Norflex)	Anticholinergic	Parkinsonism
<i>l</i> -hyoscyamine (Levsin)	Anticholinergic	Parkinsonism
physostigmine (Eserine)	Cholinesterase inhibition	Huntington's
fluphenazine (Prolixin)	Block dopamine receptors	Huntington's
perfenazine (Etrafon, Triavil, Trilafon)	Block dopamine receptors	Huntington's
mesoridazine (Serentil)	Block dopamine receptors	Huntington's
thioridazine (Mellavil)	Block dopamine receptors	Huntington's
imipramine (Tofranil)	Anticholinergic and blocks dopamine reuptake	Parkinsonism
diazepam (Valium)	Minor tranquilizer	Chorea
chlordiazepoxide (Librium)	Minor tranquilizer	Chorea

tex is also diseased, which is often so in advanced cases, weakness produced by lesions in pseudobulbar areas may add to the speech and swallowing defects which appear early in the disease process and are often severe. The difficulty in swallowing leads to aspiration, pneumonia and is often the cause of death. The disease is transmitted by an autosomal dominant gene and the onset is usually in adult life.

In 1969, Birkmayer demonstrated that L-DOPA, administered in excessive doses, produced a Huntington's-like syndrome in patients which were being treated for Parkinson's disease. He further observed that L-DOPA exacerbated the choreatic symptoms of the Huntington's patient (Birkmayer, 1969a). Following these observations, some authors (Birkmayer, 1969b, Klawans, 1970) proposed that the choreiform movements observed in the Huntington's patient are related to the activity of dopamine at receptors in the caudate and putamen. Published reports also support this hypothesis. Drugs which decrease the inhibitory action of dopamine in the basal ganglia, such as reserpine (Lazarte *et al*, 1955) and the phenothiazines and butyrophenones (Whittier, 1968; Vaisberg and Saunders 1963) are beneficial in Huntington's chorea while sympathomimetic amines, such as amphetamine (Klawans and Weiner 1974) and L-DOPA (Gerstenbrand *et al*, 1963) exaggerate the choreatic hyperkinesias. This exacerbation of symptoms by sympathomimetics is blocked by haloperidol, a known dopamine receptor antagonist (Klawans and Weiner 1974). Increased levels of dopamine are,

however, not found in the basal ganglia of patients with Huntington's disease (Chase, 1973) nor is the turnover rate of dopamine increased (Klawans, 1971; Aquilonius and Sjostrom 1971). Anticholinergic drugs (Klawans and Rubovits, 1972) also exacerbate the symptoms of the disease while anticholinesterases and cholinergic drugs reduce them (Aquilonius and Sjostrom, 1971; Klawans and Rubovits, 1972; but see also Tarsy *et al* 1973; Tarsy and Leopold, 1974). Since the disease can be treated either by decreasing sympathetic tone or increasing cholinergic actions it has been postulated that an imbalance exists between the inhibitory effects of dopamine and the excitatory effects of acetylcholine.

Other basal ganglia disorders—Gilles de la Tourette's disease is characterized by multiple tics of the facial muscles. Pathological studies indicate a deficit in the number of neuronal connections in the striatum and globus pallidus of patients with this disease. The only consistently successful therapy for this disorder has been the antipsychotic butyrophenone drug, haloperidol (Snyder *et al*, 1970). Other drugs which have been partially successful in treating Gilles de la Tourette's disease are agents such as reserpine which deplete the central neuronal supplies of the catecholamine transmitters or drugs (such as the phenothiazines) which block the actions of these transmitters at their receptor sites. The condition is exacerbated by L-DOPA and *d*-amphetamine but not by *l*-amphetamine (Snyder *et al*, 1970).

Pick's disease appears to be another disorder in which the basal

ganglia are involved. The disease has two clinical periods. During the initial period the symptoms include intellectual deterioration and memory difficulties, lapses in social conduct, disinhibition and over activity. The period of the full illness is characterized by a reduction of ideas, unusual behaviors and apragmatism, stereotypic behavior, speech reduction, and, in some cases, symptoms of damage to extrapyramidal and pyramidal brain areas. Pathology is always found in the temporal lobes of the brain, where the atrophy may be the cause of the aphasia and other speech disturbances. The basal ganglia show atrophy and proliferation of neuroglia in many cases in which there were extrapyramidal signs (Constantinidis *et al*, 1974).

Sydenham's chorea has a subacute onset of bilateral choreatic movements in late childhood. A supersensitivity to *d*-amphetamine has been documented. In addition to the above mentioned disorders, there are choreas which have been associated with systemic lupus erythematosus and a senile chorea which is characterized by pathologic alterations in the caudate and putamen. Hyperthyroid chorea has been treatable with neuroleptics which block the action of dopamine of striatal cell receptors (Klawans and Shenker, 1972). A spontaneous lingual-facial-buccal dyskinesia (Anden, 1970) also appears to be associated with pathology in the basal ganglia.

Drug induced signs of extrapyramidal dysfunction develop in nearly 25% of psychiatric patients receiving antipsychotic medications. (Crane and Naranjo, 1971,

Crane, 1973; Kazametsuri *et al*, 1972). These symptoms may occur acutely or after many months or years of treatment. The symptoms may be nearly indistinguishable from Parkinson's disease or, may have a high degree of facial-oral involvement such as grimacing and abnormal tongue movement. Acute dyskinesia is usually rapidly reversed by lowering the dosage of the antipsychotic drug while tardive dyskinesia is only slowly reversible, if at all, after drug withdrawal. Drugs inducing these syndromes include the butyrophenones, phenothiazines, thioxanthenes and reserpine.

DRUG INTERACTIONS AND CONTRAINDICATIONS

In an attempt to simplify these various basal ganglia disorders for the purpose of discussion of the pharmacotherapy as it relates to dental practice, we will consider Parkinsonism and tardive dyskinesia together, followed by athetoid and hyperkinetic disorders. Drug interactions and contraindications will be discussed.

Dyskinesias:

1. General anesthetics — Untreated Parkinson's disease may be associated with below normal blood pressure. L-DOPA treatment enhances the vasomotor depression in these patients. Hence Parkinson's patients who are untreated or who have been treated with L-DOPA may experience postural hypotension when moved rapidly from a reclining to an upright position. General anesthetic drugs, such as the barbiturate thiopental, lower blood pressure *via* medullary depression and should be used with caution since they may exacerbate the existing hypotension. Alterna-

tively, anesthetic drugs which do not by themselves reduce blood pressure are not incompatible with L-DOPA.

The dental practitioner must also remain cognizant of the fact that untreated Parkinson's patients exhibit excessive salivation which may contribute to either obstruction of the airway or aspiration pneumonitis.

2. Local anesthesia — As mentioned previously, L-DOPA used in the treatment of Parkinsonism produces orthostatic hypotension. Systemic ester-type local anesthetics (with the exception of cocaine) produce arteriolar dilation due to a direct action on the smooth muscle wall. Hence, L-DOPA-induced hypotension may be exacerbated by the inadvertent systemic administration of ester-type local anesthetic drugs. They also have a direct depressant effect on the myocardium resulting in bradycardia *via* reduced myocardial excitability and contractility. Alternatively, the antimuscarinic drugs which are used to treat Parkinsonism will block the vagal influence on the heart resulting in tachycardia. Multiple injection of local anesthetics with added epinephrine might also induce cardio-acceleration enhanced by the general stress associated with the dental procedure and thus exacerbating the effect of the anticholinergic-antiparkinsonism agents.

Systemic local anesthetics have a spasmolytic effect on intestinal smooth muscle. This effect will add to the anticholinergic effect of the atropine-like antiparkinson drugs such as bztropine mesylate, trihexyphenidyl HCl or procyclidine HCl.

3. Antiemetics — Antiemetics, such as prochlorperazine are structurally related to phenothiazines and are often used in various dental procedures. These drugs block central dopamine receptors and therefore increase the extrapyramidal symptoms of the parkinsonism patient.

L-DOPA often induces anorexia, nausea and vomiting especially in the initial stages of treatment. Dental procedures which induce the gagging reflex may potentiate the nauseated state of the patient receiving L-DOPA.

4. Analgesics — Morphine has been shown to block central receptors for dopamine (Puri and Lal, 1973; Gianutsos and Lal 1975). This blockade of dopamine receptors in the basal ganglia would reduce the inhibitory influences of the substantia nigra. In the untreated parkinsonism patient, prolonged or high dose therapy with morphine might be expected to exacerbate the symptoms or to lessen the effect of medication in the treated patient. Non-opiate analgesics have not been shown to block central dopamine receptors.

Central nervous system stimulation, mental confusion, and tremors have been reported in patients receiving orphenadrine and dextropropoxyphene HCl (Darvon) simultaneously (Hartshorn, 1970).

5. Secretagogues and antise-
cretagogues — Occasionally a dental procedure will require premedication with an antise-
cretagogue. Presently, the two most commonly used agents are atropine and propantheline bromide. Simultaneous administration of these agents to

patients who are taking anticholinergic antiparkinson drugs such as benztropine or trihexylphenidyl will potentiate the drying effect on the mucous membranes in the nasal and oral pharynxes, thus causing great discomfort and dysphagia. In addition, many patients are taking antihistamines which exhibit an anticholinergic effect thus adding to the problem. Combinations of those drugs possessing anticholinergic effects can enhance other side effects which include mental aberrations, blurred vision, constipation, and urinary retention.

6. Vitamins—Vitamin B₆ (pyridoxine) is a co-factor in the conversion of L-DOPA to dopamine in both brain and peripheral tissues. Simultaneous administration of B₆ with L-DOPA causes rapid metabolism of the latter to dopamine in the periphery, thereby reducing its availability to the brain. Vitamins containing B₆ are contraindicated in patients receiving L-DOPA. A multiple-vitamin preparation that does not contain vitamin B₆ is available (Larobec).

Choreas:

1. General anesthesia—Untreated Huntington's chorea patients may be hypersensitive to intravenous sodium thiopental given for induction of anesthesia (Davies, 1966). However, Huntington's patients are usually treated with a wide variety of drugs, as described previously. These might include fluphenazine, haloperidol, perphenazine or with phenothiazines such as chlorpromazine or with reserpine. All of these drugs have some depressant action on the CNS and may be expected to add to the depressant effects of the general anesthetics.

Antianxiety drugs or minor tranquilizers such as diazepam are frequently prescribed for patients with persistent choreas to reduce patients' symptoms exacerbated by stress. These CNS depressants may potentiate the effects of other depressants or narcotic analgesics.

2. Local anesthetics—A toxic manifestation of the ester-type local anesthetics is CNS stimulation with subsequent convulsions. The phenothiazines, especially promethazine and chlorpromazine, also have a tendency to predispose patients to convulsions. Combination of these drugs may lead to clinical difficulties in the choreatic patient who is being treated with other types of phenothiazines.

Local anesthetics with ester linkages, such as procaine, are hydrolyzed in the plasma by the enzyme pseudocholinesterase. Attempts by the neurologist to enhance the central cholinergic activity of the basal ganglia by administration of a cholinesterase inhibitor would prolong the effects of the procaine-like drugs. This interaction does not occur with the amide-type of local anesthetics such as lidocaine.

A potential interaction with the phenothiazines used in the treatment of the choreatic patient exists because of the ability of the phenothiazines to block peripheral and central α -adrenergic pressor receptors thereby producing orthostatic hypotension. Depression of the myocardium and decrease in vascular tone caused by inadvertent systemic administration of a local anesthetic will have an additive hypotensive effect with the phenothiazines. In addition, epinephrine which has both pres-

sor (alpha) and depressor (beta) effects, when injected with a local anesthetic or when used in an attempt to combat the drug-induced hypotension, may exacerbate the hypotension due to its effect on the peripheral *beta*-receptors subsequent to the *alpha*-receptor blockade by phenothiazines. Norepinephrine may be used with extreme care in larger than usual doses to maintain blood pressure.

3. Antisecretagogues—Huntington's patients treated with anticholinesterases may exhibit excessive bronchial and salivary secretions. Treatment with atropine-like agents have been shown to exacerbate the symptoms of the Huntington's patient. The use of propantheline bromide premedication for dental procedures in these patients would potentiate their symptoms and would antagonize the therapeutic effects of their cholinergic medications.

4. Sympathomimetics and stimulants—Drugs which activate central adrenergic receptors such as amphetamine tend to exacerbate choreatic symptoms. Patients with Sydenhams' Chorea have a super sensitivity to *d*-Amphetamine. Other commonly used sympathomi-

metics which exacerbate choreatic symptoms include ephedrine and pseudoephedrine.

The neurologist who treats patients with dysfunctions of the basal ganglia attempts to restore and maintain the delicate balance between central adrenergic and cholinergic neurotransmitters. In treating the bradykinetic disorders he attempts to enhance dopaminergic activity and/or decrease cholinergic activity. In the treatment of choreatic disorders the goal is to reduce dopaminergic responses or enhance central cholinergic activity. Many commonly used dental medications may upset this balance. From the above discussion, it is apparent that a thorough knowledge of the patient's disorder and pharmacological treatment of the disorder is essential to avoid serious drug interactions in the dental office. It has been estimated that up to 20% of hospital admissions are due to serious sequelae to drug interactions. Only through a good medication history and a clear understanding of those drugs commonly used by the dentist will these serious and often fatal reactions be prevented.

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Resumés of Research

ABSTRACT

Sucrose Metabolism in Resting-Cell Suspensions of Cariogenic and Non-Cariogenic Dental Plaque

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Chairman: Walter J. Loesche

A tooth which is frequently exposed to dietary sucrose will develop a carious lesion only at a site harboring cultivable levels of *Streptococcus mutans* in the dental plaque. Adjacent dental plaque will not destroy dental enamel even in the presence of the same carbohydrate substrate. The metabolic events which take place in the two types of plaque is not known. The principle objective of this investigation, therefore, was to compare sucrose metabolism of cariogenic (CP) and non-cariogenic (NCP) dental plaque collected from the same tooth and to characterize the bacterial components of each. Metabolic differences might elucidate microbial mechanisms of cariogenesis or non-cariogenesis and lead to therapeutic and diagnostic approaches to dental caries.

The plaque specimens were dispersed in buffer, divided equally

and incubated for 45 minutes with either ^{14}C -sucrose uniformly labelled in the glucosyl moiety or ^{14}C -sucrose uniformly labelled in the fructosyl moiety. Sucrose metabolism was analyzed periodically during the incubation. The contribution of the glucosyl and fructosyl moieties of sucrose to each metabolic by-product was determined. The incubation of the resting-cells and the bacteriological procedures were conducted in an anaerobic chamber at 37°C .

Radiochemical techniques were devised to analyze formation of lactic acid, soluble extracellular polysaccharide (ECP), total cell-bound and insoluble products, intracellular polysaccharide (ICP), lactic acid and from ICP catabolism, insoluble extracellular glucan (ECG), carbon dioxide (CO_2), total volatile acids, individual volatile acids, and rates of sucrose consumption. All of the metabolic

data were adjusted to the size of the plaque specimens as determined by colony forming units (CFU), Coulter Counter particle counts, and fluorometric protein analyses. Part of the calculations were performed by a computer. In addition, sucrose metabolism of pure cultures of the predominant cultivable bacteria in both types of plaque was analyzed by these techniques and compared.

Both types of dental plaque transformed from 70 to 80 percent of the consumed sucrose into lactic acid and cell-bound and insoluble products, primarily ICP and ECG. Total volatile acid production accounted for most of the remaining by-products. Clear bacteriological differences in the plaque types were observed. High levels of *Strep. mutans* in CP (averaging 40 per cent of CFU) and its virtual absence in NCP confirmed findings of earlier investigations. *Actinomyces* species dominated NCP (60 percent of CFU versus 24 percent in CP). *Strep. sanguis* levels were distinctly higher in NCP, confirming earlier reports to this effect. NCP harbored more anaerobes and

dextranase forming microorganisms than CP. Differences in the rates of sucrose consumption by the two types of plaque were highly significant. CP metabolized considerably more sucrose than NCP and consequently produced significantly higher levels of each metabolic by-product. As sucrose metabolism of a pure culture of *Strep. mutans* was similar to CP and that of *Actinomyces* species was similar to NCP, these bacteria appear to be responsible for the metabolic profile of the two plaque types.

These findings support the *Strep. mutans* infectivity concept of cariogenesis. The active consumption of sucrose by CP with rapid formation of lactic acid, ICP, lactic acid from ICP, and ECG supports theories that these metabolic by-products are important in cariogenesis. Implications of this investigation are that sufficient bacteriological and metabolic dissimilarities exist between CP and NCP to warrant development of both diagnostic and therapeutic methods based on these differences.

Summary of Research Project

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A research problem currently under study in my laboratory concerns an important group of microorganisms, the spirochetes.

The spirochetes are found throughout the microbial world and are quite prominent in the oral cavity of man and other animals. These organisms are all motile and possess an unusual structural organelle called an axial filament. This structure is considered to be homologous to the bacterial flagellum, except for its location in the cell, and therefore, believed to function as an organelle of spirochetal motility.

The studies of this particular structure center around determining as much as possible, about its chemical composition and molecular arrangement. When we have obtained this important knowledge, we can then proceed to envision how this particular structure might function as a locomotor organelle.

In order to obtain this necessary information, we must first grow these organisms in large volumes, harvest them by centrifugation and chemically remove the filaments from the cell. Once removed from the cell, the filaments are purified by a physical method, assessed for purity using the electron microscope and then subjected to a variety of analytical procedures.

It is reasonable to assume that the spirochetes depend to a great extent, on their ability to translocate as an important requirement which enables them to penetrate and invade tissue, with subsequent continued survival within the host. Therefore, an investigation concerning the structure and function of the spirochetal axial filament should provide the information necessary to understand the mechanism of spirochetal movement, which could lead to the eventual control of spirochetal translocation in oral tissues.

Research Projects Under Investigation

LEAH M. STALING, M.Sc.

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1. The Relation of Masticatory Muscle Function and Premature Occlusal Contact.

The effect of a premature 1st mandibular molar occlusal contact on the inception of muscle activity and the quantity of muscle potential generated during mastication, intercuspation and clenching is being investigated on normal occlusive subjects by recording integrated masticatory muscle EMG with occlusal contact time. (With the aid of C. Butler, Junior Dental Student, Dr. Patrick Fetchero, Restorative Dept. and David Rhoads, Electronics).

2. Mandibular Motion and Occlusal Position Control: EMG Evaluation of Mandibular-Maxillo Facial Clinical Problems.

Prosthetic problem patients presenting either neuromuscular and/or temporomandibular joint symptoms are evaluated electromyographically, using the major muscles of mastication, when mandibular motion and position control information is desired by the clinician. Determinations are: the freeway space, using a reproducible rest position obtainable by bio-

feedback; bilateral symmetry or asymmetry of intercuspation; duration of "silent periods" using the occlusograph.

3. The Practical Application of Computerized EMG of Mandibular Motion and Position to Clinical Dentistry.

Occlusal, myofunctional and/or temporomandibular joint problems presented by members of the dental student body are being screened for application of diagnostic and therapeutic techniques using computerized EMG data obtained from responses of the neuromuscular system controlling mandibular motion and position. The goal is to provide a learning opportunity as well as a diagnostic service. (With Drs. Pridgeon, Graham and Buxbaum).

4. Electrophysiologic Effect of Marine Toxins on Neuromuscular Systems (With Dr. J. Burnett).
5. Standard Method Compared with EMG-Assisted Method of Determining Rest Position of the Mandible. (With Dr. Robert Leupold and Dr. Sylvan Feldman).

Studies on Mouse Mammary Tumor Virus Produced in New Established Cell Lines

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Tissue obtained from a spontaneous mammary tumor in a mouse was processed in our laboratory to produce a primary cell culture. Electron microscopic examination of the original tumor revealed the production in the cells of numerous type B particles indicative of mouse mammary tumor virus (MMTV). Upon continued serial passage of the cells *in vitro* a new established cell line developed which has now been passaged more than 100 times over the past two years.

Our primary research interests in this cell line are two-fold. First to characterize these cells as to their optimal growth kinetics and morphology. Second, to determine if these cells are still producing mouse mammary tumor viruses;

if so, these cells will serve as an excellent *in vitro* model to study many aspects of tumor virus/cell interaction.

Preliminary evidence that the cells are producing MMTV has been obtained. Namely, the cells produce positive immunofluorescence in an indirect fluorescent antibody staining procedure using MMTV-specific antibody, and C^{14} -labeled material sediments at the density of MMTV in sucrose density gradients. We are now attempting to identify the virus directly by electron microscopic examination of: whole cells and thin section cell samples, and samples prepared by concentration and purification of material from the supernatant fluids.

Studies of Gastric Acid Secretion

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College of Dental Surgery, Dental School, University
of Maryland at Baltimore, Baltimore, Md. 21201*

Primarily investigations of energetics of H^+ transport in frog gastric mucosa. Recent studies add kinetic evidence that H^+ and Cl^- transport systems are independent (J. Baltimore Coll. Dent. Surg. 29:51, 1974), show that previous *in vitro* preparations were CO_2 -limited (Am. J. Physiol. 227:300, 1974) and that properties are somewhat different at high CO_2 (Am. J. Physiol. 228:928, 1975). At slightly acid serosal pH, an interesting sudden dip in potential difference and fall in resistance occur under anoxia (Am. J. Physiol. 230:61, 1976) which seems to be due to increased H^+ permeability (Kasbekar, et al., Eds. "Recent Developments in Gastric Secretion," in press), and may be related to the etiology of stress ulcer. These sudden changes can be pro-

duced by substrate deprivation as well as anoxia, and are not reversed by ATP. (Biophys. J. 16: 130a, 1976). Studies on the oxygenation of the frog mucosa (Am. J. Physiol. 229:1510, 1975) and of CO_2 and O_2 supply to the similar mucosa of the dogfish (Bull. Mt. Desert I. Biol. Lab. 14:58, 1974 and 15: in press) have been carried out during the summer and collaborative studies on the morphology of the frog gastric mucosa with Dr. R. M. Meszler (Anat. Rec. 178:415, 1974 and mms. in preparation) and with Dr. R. K. Nauman of the cytochromes of a microorganism (Abst. 75th Ann. Meg. Am. Soc. Microbiol. p. 133) have been conducted. Research supported by grants from NSF. (PCM-73-06699 & PCM-75-23471)



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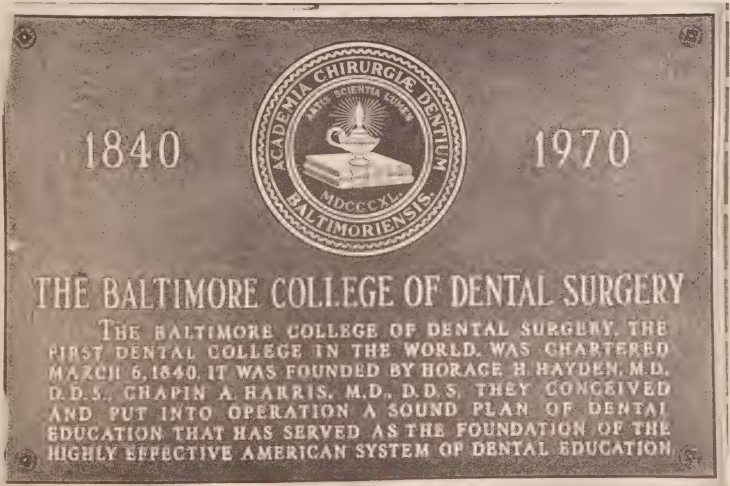
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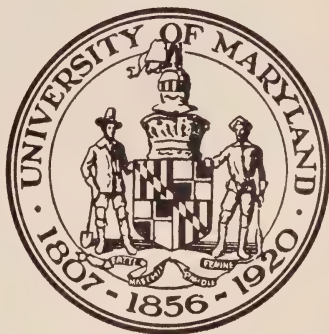
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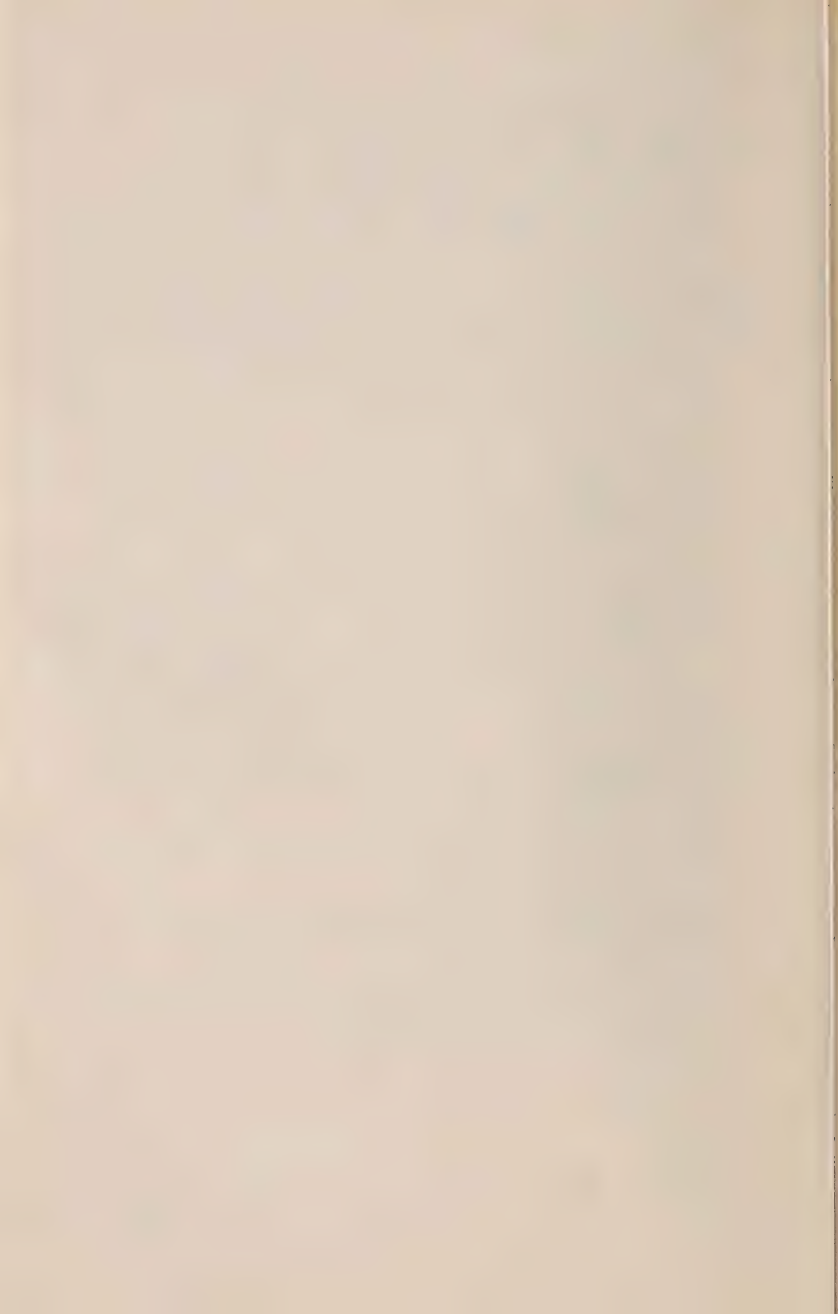


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Induction of Cleft Palate by X-Irradiation of Prenatal Mice

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Induction of Cleft Palate by X-Irradiation of Prenatal Mice

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SUMMARY

Pregnant CD1 mice were irradiated on the 12th gestational day with 400 rads of 250 KVcp X-rays at a dose rate of 102 rads per minute. All fetuses examined on days 14-20 of gestation were noted to possess clefts of the secondary palate. Measurements of CR length, head diameter and cleft distances indicated that cleft palate may have been due to inhibition of orofacial growth.

INTRODUCTION

Clefts of the lip and/or palate constitute one of the most common craniofacial birth defects in humans (13% of total). Their incidence among live born Caucasians may exceed 1/600, provided that allowances are made for underreporting (Meskin and Pruzansky, 1967). It is known that these anomalies may be induced in experimental animals by nutritional deficiencies, such as those of folic acid and Vitamin B₁₂ as well as administration of teratogens such as salicylates, chlorcyclizine, 6 aminonicotinamide, excess Vitamin

A, cortisone and ionizing radiation (Kraus, 1970; Pruzansky, 1973; Walker and Frazer, 1956, Russell and Russell, 1954; Ross and Walker, 1963; Gartner, Hiatt and Provenza, 1977). The exact mechanism whereby these agents induce cleft palate is poorly understood. In fact, experimentally induced clefts of the palate may differ in their mode of formation from spontaneous clefts due to the presence of multifactorial threshold systems operating during elevation, approximation and/or fusion of the mammalian palatal shelves (Kraus, 1970).

The purpose of the present investigation was to study the effects of X-irradiation on the palatal closure in mice and the interrelationships of various measurable parameters during the formation of the secondary palate.

MATERIALS AND METHODS

Animals used in this investigation were the progeny of timed pregnant CD1 mice (Jackson Laboratory). The pregnant females were divided into an experimental

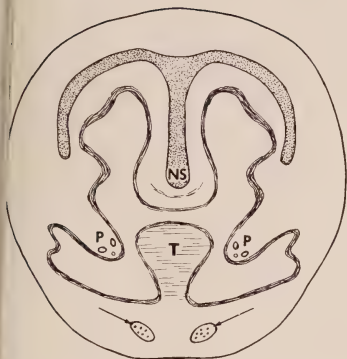


FIGURE 1. Drawing of a cross section of a cleft palate mouse embryo, seventeen days postconception. Note the lateral palatine shelves (P) of the maxillary process, the median nasal septum (NS) as well as the tongue (T) and Meckel's cartilage (arrows).

and a control group. The experimental group was irradiated whole body, with 400 rads of 250 KVcp X-rays at a rate of 102 rads per minute for a total time of 3.92 minutes on the 12th gestational day, where insemination is designated as day 0. Pregnant irradiated and control mice from 14 to 18 days of gestation were sacrificed by chloroform inhalation. The fetuses were obtained by laparotomy and, when size permitted, the fetuses were decapitated. Severed heads or whole animals were immediately placed on the rapid freeze bar of a Lipshaw Cryotome, permitting ample freezing times of 15 to 20 minutes. Frozen heads were oriented snout to the chuck so that sections were recovered in a frontal plane perpendicular to the secondary palate. Sections 12 micrometers thick, were placed on clean glass slides and removed from the

environment of the cryostat for microscopic evaluation. All slides were stained with Saffarin O.

The tissues were dehydrated in a series of graded alcohols, cleared in xylene and mounted in Permount. Additionally, animals and/or severed heads were fixed in 10% neutral buffered formalin or Bouin's fixative and were embedded in paraffin. Five micrometer thick serial sections were either stained with hematoxylin and eosin or according to the periodic acid Schiff technique.

RESULTS

Administration of 400 rads of X-rays resulted in the formation of clefts of the secondary palate in all fetuses. Measurements were made of the space between the tips of the palatine shelves (Fig. 1), of the head diameter (Fig. 1) in fixed material, as well as of the crown-rump length of all fetuses. A comparison of the distance separating

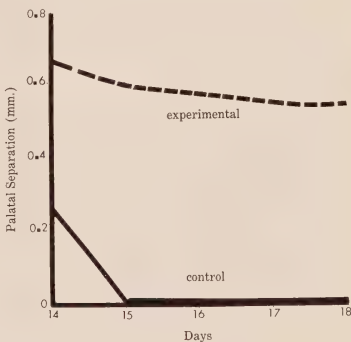


FIGURE 2
Comparison of Palatal
Separation
by days

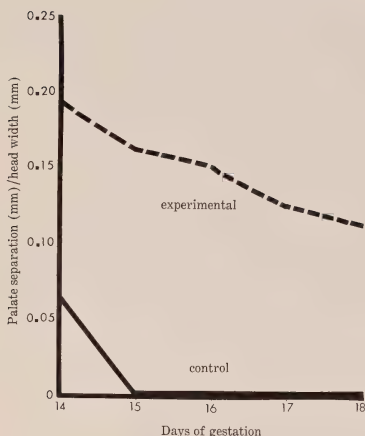


FIGURE 3
The ratio of palatal separation
to head width as
a function of
gestation

the tips of the palatal shelves in the irradiated animals as a function of age demonstrated a decrease of 0.07 mm during the four day interval (Fig. 2). The ratio of palatal separation to head width also demonstrated a decrease, from 0.195 to 0.110 in the irradiated fetuses (Fig. 3). The greatest alteration was noted in the crown rump length of the fetuses, where the control animals were much larger than their irradiated counterparts. The slopes of the growth curves differed radically (1.12 vs. 0.50) for the two groups of fetuses (Fig. 4). This not only indicated that the irradiated animals did not reach normal size but also that they developed at a slower rate.

The secondary palate of the normal, unirradiated mouse was fused by the 14th gestational day, except

for the posterior region (Figs. 5, 6, 7), where the shelves were elevated but still separated by a space. The epithelium of the palatal shelves were two to three cell layers in thickness (Figs. 6, 7) and the palatine arteries were near the tip of the shelves (Fig. 7). The shelves of the 14 day old prenatal irradiated mouse were still in the vertical position (Fig. 8). On the 15th prenatal day the primary palate of the control animals was in the process of fusion (Fig. 9), while the secondary palate was completely established (Fig. 10). The vomer and maxilla bones were well developed (Fig. 10). In the irradiated fetus, the lateral palatine processes were still in the vertical position, the vomer was not well developed while maxillary

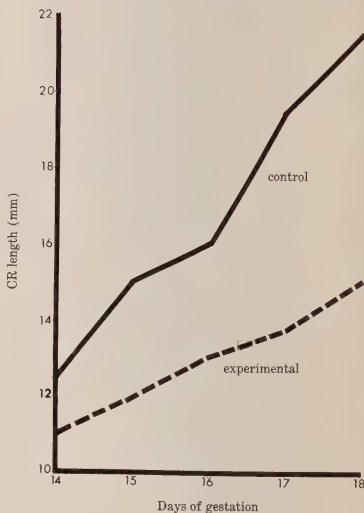


FIGURE 4
Crown-Rump length
as a function of gestation

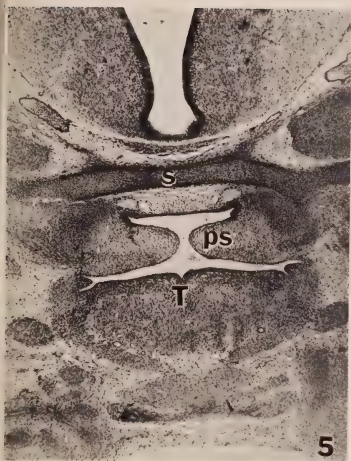


FIGURE 5. Posterior palatal region of a 14 day old, unirradiated mouse fetus. Note the unfused lateral palatine shelves (PS), the base of the tongue (T) as well as the base of the skull (S). $\times 41$

osteogenesis was in the initial stage of development (Fig. 11). The palatine arteries were relatively well established in the irradiated 16 day prenatal animal (Fig. 12). The palatine shelves were vertical in some fetuses (Fig. 12) and horizontal in others. Meckel's cartilage was poorly developed, while the vomer was beginning to be established (Fig. 12). The palatine shelves of the 17 day prenatal fetuses were not fused (Fig. 13). The vomer and maxilla bones were well developed as were the palatine shelves (Fig. 13). The tongue, as in all irradiated fetuses, was slightly anomalous, not only in the development of the internal musculature but also in its external morphology (Fig. 13).

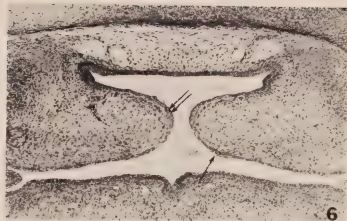


FIGURE 6. Higher magnification of Figure 5. Note the mitotic figures in the epithelium of the shelf (arrow) as well as the thickened region of the epithelium at the medial ridge (double arrows). $\times 73$

DISCUSSION

The appearance of cleft palates in laboratory animals and humans is believed to be due to a combination of genetic and environmental forces (Kraus, 1970; Langman, 1975; Gartner, Hiatt and Provenza, 1977 and DePaola, 1976). The results of the present study confirms those of previous investigators (Callas and Walker, 1967; Russell and Russell, 1954), indicating that ionizing radiation administered at a specific period of gestation prevents palatal fusion. urements involving the crown-

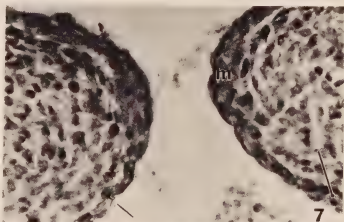


FIGURE 7. Higher magnification of the epithelial region of the previous figure. Note the basement membrane (arrow), the thickened epithelial covering of the medial ridge (M) as well as the mitotic figure (pointer). $\times 290$

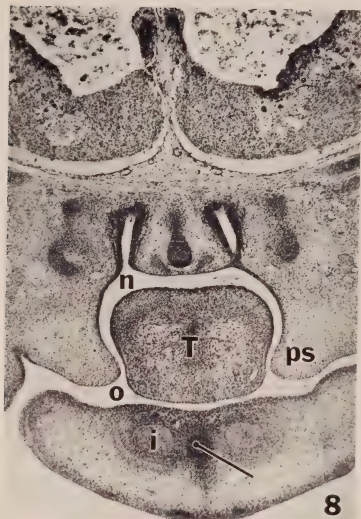


FIGURE 8. Irradiated, 14 days prenatal fetus. Note that the lateral palatine shelves (PS) are in the vertical position and the tongue (T) is occupying both the oral (O) and nasal (N) cavities. The mandibular incisors (I) and Meckel's cartilage (pointer) are also evident. $\times 41$

The exact mechanism by which radiation causes this defect is not known.

The role of the tongue and other orofacial musculature in palatal closure has been considered, but Jacobs (1971), utilizing muscle-paralyzing agents administered at the time of palatal closure, was unable to produce clefts. Ross and Walker (1963), demonstrated that the movement of the palatal shelves from a vertical to a horizontal plane occurred even when the tongue was excised, but the shelves were separated further from the nasal septum than when

the tongue was in place. The radiation dose of the present experiment debilitated the development of the tongue which probably further restricted the upward swing of the palatal shelves. One of the major points of interest indicated by the present experiment was that the distance separating the palatal shelves of the irradiated animals remained almost constant for the duration of the study, suggesting that radiation inhibited normal growth of the lateral palatine shelves. Such inhibition of normal growth was also evident in meas-

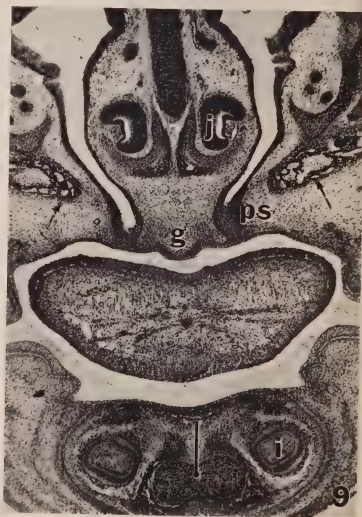


FIGURE 9. Unirradiated 15 day old fetus. Note the fusion of the globular process (G) with the lateral palatine shelves (PS) of the maxilla. Jacobsen's organs (J) are well developed. The maxilla bones are in the process of formation (arrows) and the mandibular incisors (I) are well established. Also note Meckel's cartilages (pointer) fusing in the midline. $\times 41$

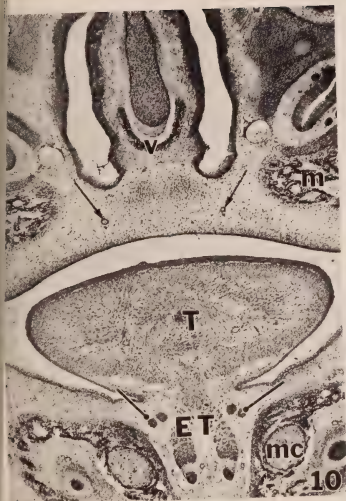


FIGURE 10. Fifteen day old, unirradiated mouse fetus. Note the palatine arteries (arrows), the well formed vomer bone (V), the maxillary bones (M), and the tongue (T). Meckel's cartilages (MC) and the extrinsic musculature of the tongue (ET) are both well established. Also note the ducts of the salivary glands (pointers). $\times 41$

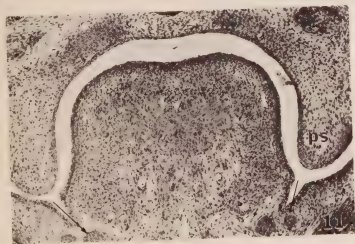


FIGURE 11. Fifteen day old, irradiated fetus. Note the absence of the vomer bone, the vertical position of the palatine shelves (PS) as well as the poorly developed tongue. Arrows indicate the ducts of the salivary gland. $\times 73$

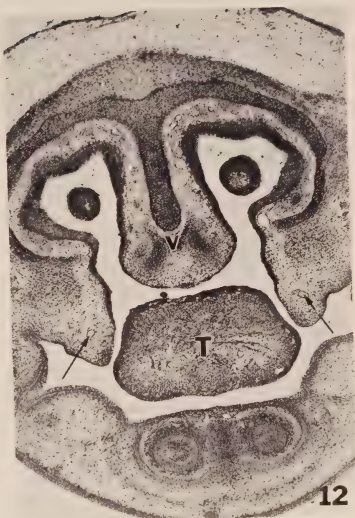


FIGURE 12. Sixteen day old irradiated fetus. Note the beginning of the development of the vomer bone (V), the well developed palatine arteries (arrows) of the vertical palatine shelves as well as the poorly developed intrinsic musculature of the tongue (T). $\times 41$

rump length of the experimental fetuses, which lagged about three days behind their unirradiated counterparts.

Ionizing radiation, then, appears to inhibit normal growth of the fetus as well as impair normal development of the oro-facial region. Although its *modus operandi* is not understood, its continued study will permit an elucidation of the mechanisms underlying the spontaneous occurrence of cleft palates.

ACKNOWLEDGMENTS

The authors wish to thank Miss Vilma Pascual for her photographic



FIGURE 13. Seventeen day old irradiated fetus. Note the horizontal position of the palatine shelves (PS) and the palatine arteries (arrows). The tongue (T) is somewhat malformed and its extrinsic and intrinsic musculatures are poorly developed. $\times 41$

assistance and Mrs. Elizabeth Lewis for typing the manuscript. Our thanks are also extended to the Radiation Therapy Department of the Medical School of the University of Maryland for administering the radiation exposure under the auspices of grant CA 06518-11, National Cancer Institute.

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The Dent-Pharm Therapeutics Program: An Interdisciplinary Approach in Dental Education

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SUMMARY

1. The Dent-Pharm Therapeutics Program, jointly sponsored by the University of Maryland Schools of Dentistry and Pharmacy, is a project whose purpose is to explore patient-oriented, interdisciplinary education of health professionals.

2. The Dent-Pharm Therapeutics Program provides a dual approach to the interdisciplinary training of future dental and pharmacy practitioners.

a. As part of required undergraduate courses, all dental and pharmacy students receive lecture and written material to acquaint them with issues related to each other's professions.

b. In addition to the required course material, special study

electives have been established to allow selected dental and pharmacy students an opportunity to gain in-depth knowledge of the other's profession.

3. The Dent-Pharm Therapeutics Program is attempting to create a role model of the working relationship that can exist between dentists and pharmacists in areas of patient care. This goal is being accomplished by way of clinical pharmacy consultations concerning dental drug therapy, in addition to the traditional pharmacy services of drug control and distribution in the dental school.

4. The Dent-Pharm Therapeutics Program provides an opportunity to compile and generate information concerning drug utilization in dentistry and promotes collaborative research between the schools of dentistry and pharmacy.

INTRODUCTION

Since the early 1970's, several schools of dentistry and pharmacy have been involved in developing co-sponsored teaching programs for their students. A recent survey which was conducted by the staff of the University of Maryland Dent-Pharm Therapeutics Program indicates that some twenty-two such programs currently exist. The major emphasis of these established programs has been a sharing of faculty, students, and lecture time, and because each program has evolved independently without guidelines, the areas of emphasis vary greatly from campus to campus. Thus, some schools are committed to joint research (Hamner, 1971), while others have progressed to singular areas of patient care such as non-prescription dental products (Nelson, 1976), drug distribution (Wertz and Brookreson, 1972), and clinical pharmacy drug consultation (Schlegel, Pallasch and Oksas, 1973).

The Dent-Pharm Therapeutics Program was established at the University of Maryland in July, 1974. It was initially funded by a Health Professions Special Project Grant for five years. Like similar ventures, its main objective was to develop between the Schools of Dentistry and Pharmacy a cooperative education program which would emphasize increased awareness of each profession's health service capabilities and promote the health team concept. The Dent-Pharm Therapeutics Program is unique in that it employs successful features of other projects in a comprehensive and organized framework while adding innovative aspects not found in similar ventures (Griffenhagen, 1976).

Table 1 provides a schematic representation of the components of the Dent-Pharm Therapeutics Program.

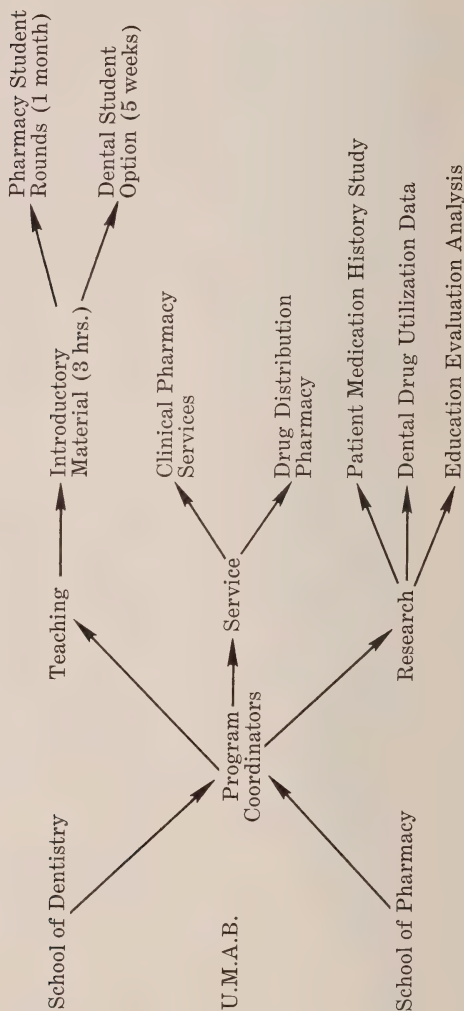
EDUCATIONAL METHODOLOGY

In an effort to provide interdisciplinary training to future dental and pharmacy practitioners, a two-fold teaching technique is utilized. This approach consists of imparting basic introductory material to as many students as possible as well as providing in-depth information to students on an elective basis.

All dental and pharmacy undergraduate students receive material, as part of required course work, to acquaint them with issues related to each other's profession. This introduction consists of a discussion of educational training and a description of specialized practice and patient services in each field. Pharmacy students are taught about proper selection of dental over-the-counter items and their health education role in preventive dentistry, while dental students learn about the pharmacists' criteria in drug product selection and the pharmacists' role in drug monitoring and consultation. These introductory lectures are given to the junior and senior students in the respective schools and are about three hours long.

In addition to the introductory material mentioned above, the pharmacy student may elect to participate in a dental clerkship rotation provided by way of the Professional Experience Program (PEP) in the School of Pharmacy. In this way the pharmacy student may gain an in-depth knowledge of dental practice. A wide variety of

TABLE I
Components of the University of Maryland
Dent-Pharm Therapeutics Program



experiences and teaching techniques (i.e. self-instruction syllabus, competency based learning, etc.) are used in the dental clerkship. The rotation begins with a pre-clinical seminar in which the pharmacy student learns dental terminology, treatment, and drug therapy. This seminar is followed by two weeks of participation at the dental school with half-day sessions devoted to clinical pharmacy services to dental patients and observation in various clinical areas such as Oral Diagnosis, Periodontics, Oral Surgery, and the Special Patient Clinic. The pharmacy student completes his rotation in a community pharmacy setting where he is expected to use his newly gained knowledge of dentistry. Finally, the pharmacy student is required to write a short research paper on some aspect of dental drug therapy.

The dental student has an opportunity to learn more about pharmacy practice by way of five, two-hour pharmacy rotations in diverse areas of specialized pharmacy practice. These areas are community pharmacy, hospital pharmacy, clinical pharmacy, nuclear pharmacy, and a poison information center. Based on dental student evaluation of the pharmacy rotation, an area of drug manufacturing will be included in future pharmacy rotations. The dental student receives no credit for his voluntary participation in the pharmacy rotations, although each student is awarded a certificate of appreciation for this attendance.

SERVICE METHODOLOGY

The Dent-Pharm Therapeutics Program has attempted to create a model of the working relation-

ship that can exist between dentistry and pharmacy in areas of patient care. This has been accomplished not only by having teaching staff available for formal clinical consultation regarding specific cases, but also by providing direct services to dental patients on a routine basis.

The pharmacy services conducted at the dental school include newer aspects of clinical pharmacy practice as well as traditional distributive functions. Pharmacy consultation concerning dental drug therapy is achieved by way of patient medication interviews conducted periodically on comprehensive care patients screened in the Department of Oral Diagnosis. These drug history interviews include questions about previous health status, current illness, prior response to medication, and present drug regimen. The resident clinical pharmacist then enters in the patient's record appropriate dental drug therapy recommendations which would help to prevent adverse drug reactions.

The newest area of service involvement has been planning and construction of an in-house dental pharmacy to aid in education and to provide needed patient service in the dental school. The dental pharmacy is an excellent drug information center for dental students and it also provides an ideal area for the pharmacy student to learn how prescription and over-the-counter items are used in dentistry. The dental pharmacy also serves an important role as the central location in the dental school from which all clinically useful drugs may be ordered, stored, and controlled.

RESEARCH POTENTIAL

While the basic aim of the Dent-Pharm Therapeutics Program is cooperative education, there is also an obligation to compile and generate information about drug utilization in dentistry. In addition to data gathering, it is hoped that the program will stimulate independent collaborative research ventures among various departments at the two schools.

Currently the program staff is evaluating the program's service and educational components. The patient medication history information is being computerized for gross analysis of drug problems encountered in dental patient populations. Another study will be conducted to measure the impact the dental pharmacy has on the prescribing of drugs at the dental school.

In terms of educational evaluation, student surveys have already been helpful in making curricular modifications to make the interdisciplinary teaching aspects more productive.

DISCUSSION AND FUTURE CONSIDERATIONS

As presented in this paper, the Dent-Pharm Therapeutics Program has grown rapidly during its first two years. It has proven that interdisciplinary teaching and cooperation is not only desirable, but is also achievable. It now appears that the provision of clinical pharmacy services creates a favorable environment for teaching students while generating data for applied research.

The results to date prove the value of the program. Some 180 students at both schools have received the introductory lectures about Dental-Pharmacy cooperation. An additional 10 dental students and 5 pharmacy students have participated in the elective advanced training in this area. Additionally, 26 faculty members serve on a part-time basis as consultant-instructor in the teaching program.

In terms of patient care, drug consultation has been shown to be invaluable to both students and faculty. As shown in Table II, these consultations are inquiries directed to the Dent-Pharm Therapeutics staff before dental prescribing or treatment is rendered. This drug information consultation seems to be gaining acceptance and will be pursued further when the dental pharmacy is fully operational in February of 1977.

Possible areas of future development are also being explored and discussed. For example, the program can further promote community practitioner interest by sponsoring continuing education programs for both groups. Similarly, it is hoped that future areas of teaching would include graduate student seminars in applied clinical pharmacology. Relating to the area of service, the program plans to establish a Pharmacy and Therapeutics Committee, comprising faculty from both schools, to review dental drug utilization policies in the dental clinic. If successful, this project could serve as a model for similar cooperation at the local professional association level.

TABLE II
Clinical Pharmacy Consultations Provided
by the University of Maryland Dent-Pharm
Therapeutics Program
(6 months—1976)

Type of Information Requested	Dental Student	Dental Faculty/ DDS	Pharmacy Faculty/ RPh	Pharmacy Student	Total Inquiries
Therapeutic Management of Dental Patient with Medical Problems.....	3	6	0	2	11
Drug Identification.....	6	4	0	0	10
General Questions About Product Information	2	3	1	1	7
Pharmacy Law and Prescription Writing.....	4	1	1	0	6
Previous Drug Allergy and Toxicity.....	6	3	0	0	9
Selection of Drug of Choice Indicated.....	2	2	1	0	5
Pharmacosedation.....	8	2	0	0	10
Dosage Form and Posology..	2	1	0	0	3
Management of Drug Induced Toxicity.....	0	0	2	1	3
Dental Treatment and Services.....	0	0	3	0	3
Drug Interactions.....	2	0	0	0	2
Drug Side Effects.....	0	1	1	0	2
Rationale of Dental Prescribing; Product Selection.....	0	0	2	0	2
Total Consultations	35	23	11	4	73

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Note to Editor

**Dental Student Admissions
Baltimore College of Dental Surgery
Dental School
University of Maryland at Baltimore**

CHARLES B. LEONARD, JR., Ph.D.
*Director—Office of Dental Admissions
Baltimore College of Dental Surgery, Dental School
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Note to Editor

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The Admissions Committee of the Dental School is composed of three subcommittees: Dental Student Admissions, Dental Hygiene Admissions, and Postgraduate Admissions. The Dental Student Admissions Subcommittee is composed of three basic science faculty, three clinical science faculty, two dental students plus four alternates, one non-faculty alumnus plus an alternate, the Chairperson of the Minority Recruitment Committee as an ex-officio member, and the Director of the Office of Admissions as the Chairperson. This subcommittee presents a report to the Faculty Assembly of the Dental School to inform the members of the policies and procedures followed when an applicant applies to and is accepted by the Dental School. This report is the subject of this article for those who may also be interested in the work of the Subcommittee on Dental Student Admissions.

The Committee has three general policies, namely to offer equal education opportunities to all persons of all ethnic backgrounds, to consider the application from children of alumni by resident standards for the purpose of admissions, and to attempt geographical representation of the qualified applicants applying from the State of Maryland. Two general conditions, which must be fulfilled by each applicant by June 30th of the year in which the applicant enters dental school, are that all requirements must be completed and that all unabsolved conditions and failures must be resolved.

The first of five specific application requirements is that the applicant must have a minimum of 90 credit hours (exclusive of physical education and military science), of which a maximum of 60 credit hours may come from an accredited Junior or Community College.

The second specific requirement is the applicant must have six credits in English and eight credits including laboratory in Biology (Zoology), Inorganic Chemistry, Organic Chemistry, and Physics. Additional science courses which are usually suggested for an applicant include Biochemistry, Cellular Biology, Comparative Vertebrate Morphology, Genetics, Microbiology, and Physiology. The third specific requirement is favorable recommendations from either a predental committee or, if this is not available, one recommendation each from a Biology and a Chemistry teacher. The fourth specific requirement is acceptable Dental Admissions Test (DAT) scores where the minimal DAT scores for non-residents must be scores of 5 or greater in the Academic Average, Two Dimensional Perceptual Motor Ability Test (2D-PMAT), and Three Dimensional Perceptual Motor Ability Test (3D-PMAT) sections of the test while those for residents are scores of 4 or greater in these same areas. The final specific requirement is acceptable GPA values where the minimal GPA values for non-residents must be values of 3.3 or greater for the Science and Cumulative GPAs while the preferred GPA values for residents are values of 2.6 or greater in these same areas.

Each applicant is required to take the DAT as a part of their evaluation procedure. This test is given at various testing centers on specific dates and at a specific location throughout the United States and Puerto Rico, which are listed in the pamphlet describing the test. An application for the test is sent to the applicant upon specific request or as a part of the applica-

tion packet. The preferred date to take the test is in April of the year prior to the desired date of admission.

There are five steps involved in applying to our Dental School. First, the applicant requests application material from the Office of Admissions after May 1 of the year prior to the date of desired entrance into dental school. The packet includes a catalogue, DAT information, initial application instructions and a request card to send to the American Association of Dental Schools Application Service (AADSAS) for the application forms. The second step is to return the completed application to AADSAS as soon as possible with the latest date for receipt of applications being December 1. The purposes for returning these applications to AADSAS are to duplicate the application in order to send them to schools selected by the applicant, to verify the applicant's coursework which has been coded by the applicant from official transcripts, and to provide a statistical analysis of all applicants applying to a given dental school. As a result, a folder containing xerox copies of the applicant's official transcripts, answers to specific questions, and other information such as undergraduate course work taken, GPA values, DAT scores, etc., are forwarded to each dental school selected by the applicant.

The third step is a review of each folder received from AADSAS by the Director of Admissions and, if necessary, by the Admissions Committee. The purpose of this is to determine whether the minimal non-resident and preferred resi-

dent standards have been met. As a result of this review, either preliminary rejections are sent, if standards are not met, or a Maryland packet containing a Maryland application, recommendation forms, and final instructions for completing the application is issued to the qualified applicants.

The fourth step in the application procedure is to post the returned Maryland application with the Office of Registrar for the purpose of determining the residency status of the applicant by the registrar.

The final step is an interview before the Admissions Committee. All qualified applicants, who are Maryland residents as well as non-resident children of alumni, and some highly qualified non-residents are scheduled for an interview. The number of non-residents is restricted since only 15% of our entering class can be non-residents. Present at the interview are two faculty members, one non-faculty alumnus, one student member, the Chairman of the Minority Recruitment Committee, and the Director of the Office of Admissions.

After the interview, each committee member rates the interview as superior, good, fair, or poor taking into consideration such factors as appearance, manners, personality, responses to questions, ability to communicate, and motivation. The initial status of the applicant regarding the desirability of an offer of enrollment is made by majority vote if all data is available. Otherwise, the applicant is placed in an active hold until all data has been compiled for a decision.

A ranking of each applicant interviewed is made to indicate the relative standing of applicants based upon the criteria involved using the parameters of the Science GPA, the Cumulative GPA, the Academic Average of the DAT, and the PMAT Average of the DAT. Adjustments are made for 2D-PMAT and 3D-PMAT scores of the DAT, which are below the national average, and for the average interview score where ratings of superior, good, fair and poor are equated to a 4-3-2-1 scale.

The earliest date for offers is December 1 where the source of candidates would be the pool of applicants who had received a superior status as a result of the interview and other factors. The latest date for offers is approximately the second week of the fall academic session where the source of these candidates would be those on the alternate list. At the present time, those candidates on the alternate list, who do not gain entrance, are guaranteed a place in the class for the following year.

The method utilized to select candidates is by majority vote of the Admissions Committee upon final review of each qualified applicant. Approximately one-fourth of the class is offered as of December 1 with another one-fourth offered around January 31. The remaining spaces are filled after all qualified applicants have been interviewed. In addition to an applicant's relative standing in the ranking, other factors such as residency status, minority status, alumnus status, Maryland geographical status, improvement in the undergraduate courses work, and factors perceived during the interview are considered in selecting candidates

for entrance. The candidate has thirty days to reply to the offer if the applicant was offered before April 1 and fifteen days if offered after April 1. A non-refundable deposit of \$200 is required at the time of acceptance for the purpose of insuring registration in the class and is credited towards tuition cost.

There are three types of requests for advanced standing for dental admissions. One type is from students currently enrolled in United States and Canadian dental schools. The procedure involved is to determine if the applicant initially met admission requirements of our dental school on the basis of the applicant's undergraduate GPA and DAT scores at the point in time when the applicant made application to the dental school presently attending. If the applicant would have met entrance requirements, a Maryland application is sent and an interview is scheduled. Documents in support of the applicant's request for transfer are requested to determine if the applicant was eligible to advance to the next higher class in the dental school presently attending, to determine if the requirement for achievement of an overall "C" average in all work excluding Basic Dental Sciences and Biomedicine, which must be a grade of "C" or better, and to determine if the applicant has a letter of honorable dismissal and recommendation from the Dean of the dental school presently attending.

If a transfer applicant meets these requirements for admission, all information is forwarded to the appropriate Advancement Commit-

tee for review and recommendation to the Admissions Committee regarding the eligibility of the applicant for advanced standing. The final step is to notify the applicant that either the request for advanced standing has been rejected or that it has been approved, subject to availability of space and to course requirements as recommended by the Advancement Committee.

A second type of request for advanced standing comes from students currently enrolled in foreign dental schools. The procedure involved is to inform the applicant to apply as a regular applicant seeking admission to the first year class entering in September. It would be extremely difficult to assess the curriculum of a foreign dental school to the point where credit could be given for those courses compared to those in our curriculum.

The third type of request for advanced standing is from graduates of foreign dental schools. The procedure in this case is to refer them to the Secretary of the Maryland State Board of Dental Examiners for the purpose of taking the licensure examination to practice in the State of Maryland. If the licensure attempt fails, the applicant is informed that application to our institution must be made as a regular applicant seeking admission to the first year class. These applicants are also sent a list of dental schools which admit graduates of foreign dental schools with advanced standing.

Future activities of the Dental Student Admission Subcommittee include the following: conduct a

study to determine the correlation between various parameters in the dental student's undergraduate record and performance in specific courses or at the end of specific years of the student's dental education for the purpose of assisting the Admissions Committee in selecting qualified applicants; the utilization of a structured interview format for each applicant for

the purpose of becoming more objective in assessing an interview when considering equally qualified applicants as judged by GPA values and DTA scores; and participate in a validation study of a Clinical Analysis Questionnaire being conducted by the American Dental Association for possible inclusion in future Dental Admissions Testing program.



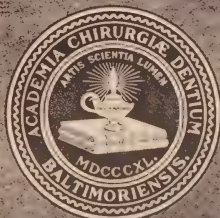
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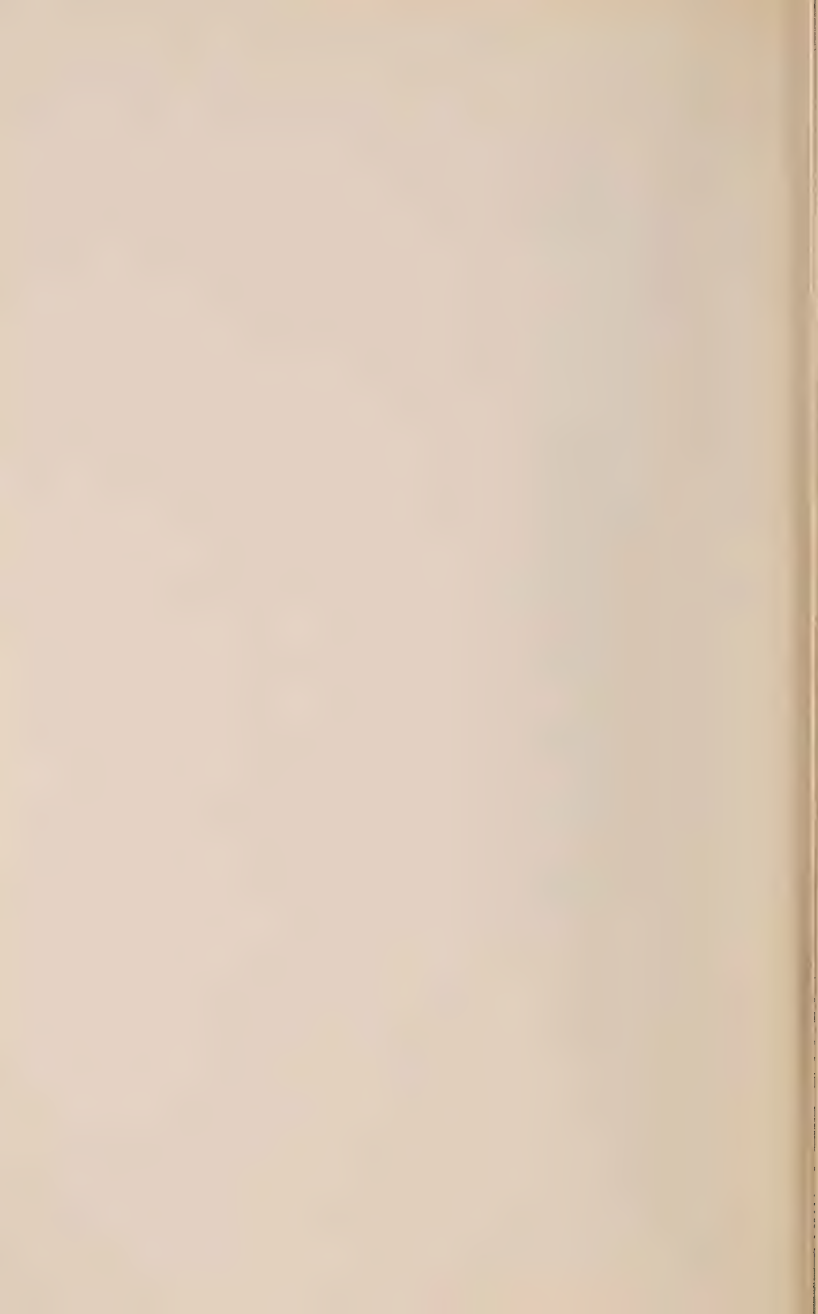
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Modern Concepts and Trends in Dentistry

JEANNE C. SINKFORD, D.D.S., Ph.D.*

Modern Concepts and Trends in Dentistry

JEANNE C. SINKFORD, D.D.S., Ph.D.*

Dentistry has evolved as a profession with the conceptual tripod that includes education, research and service as inseparable, essential components. We have reached a moment in history where federal legislation, consumer demands for service and accountability will have significant influence on our concepts and trends in the future. The health planners, educators and health care deliverers will be challenged to unify in strategies that will affect the overall quality of health and systems of health care delivery in this country.

Our purpose and dedication in Dentistry and in health today include the concept that health care is a right for all people and not only for a privileged few who may be able to afford it. Also, concern for patient access to health services, health education, attitudes toward health and life, today complicate what *used* to be the primary mission of the health professional: to alleviate pain, eliminate disease and to prolong life.

Dentistry has evolved as a crisis oriented profession and most dental care systems have been designed to relieve pain; that is, to extract or to drill and fill. We have just begun to focus on preventive concepts that would reduce the magnitude of oral

disease to a level where health care "may" become possible and accessible to all.

While this is our mission and our present dream, we must look at the current facts. In the United States, we are a far cry from solutions for the oral health problems that beset our nation as we review such statistics as: half the children between ages 4 and 14 in the United States have never seen a dentist for treatment; there are over 1 billion unfilled dental cavities and half of the school age children have some form of malocclusion and gingival disease;¹ oral cancer strikes some 14,000 persons annually and causes 1 in every 40 deaths from cancer;² twenty-five million adults have lost all their teeth by middle age and another 25 million have lost half of theirs;³ 6,000 babies are born annually with cleft lips or cleft palates.⁴ In addition, dental bills of American families exceed 8 billion dollars annually despite the fact that less than half the population visits a dentist's office each year.⁵ And—American industry loses over 100 million man hours of production time annually because of problems related to dental health.⁶ Our overall dentist to population ratio is 1:2,500 whereas in the black community, the ratio is 1:12,500.⁷

* Professor and Dean, Howard University College of Dentistry. Gaver Memorial Lecture, Baltimore College of Dental Surgery, Baltimore, Maryland, May 12, 1977.

Our maldistribution problem further complicates the picture since, in that United States, dentists are concentrated in urban areas which leaves large segments of the population in underserved, rural and other short-age areas.

The current system of health in the U.S. is large and costly. It utilizes about 4.5 million people—double the number it employed 25 years ago—making it one of the largest industries in the country. Spending for health care in the U.S. now totals in excess of \$100 billion annually or about 8% of the gross national product!⁸

In the U.S. our current health status has been complicated by the response to increased social pressures and federal support of the concept of access to health care as a right for all people. The U.S. has attempted to make health care available to all through support of *some* form of a health insurance program (we do not have a national health insurance), Health Maintenance Organizations, Neighborhood Health Centers, Third Party Payment Plans, etc. Although these efforts are being made to improve health care delivery *when* and *where* it is needed, we still operate without a clear cut national health policy since our most effective planning has been on a local or regional basis. The Health Planning and Resources Development Act of 1974⁹ provided federal funding for regional health planning and development. Also, the new Health Manpower Bill¹⁰ passed in 1976 will give support to health educational training and will attempt to correct maldistribution of dentists and physicians in remote areas of the United States.

As we look at the progress dentistry has made through the years, we can be proud that as a health profession, dentistry has undergone *steady* growth since the founding of the first dental college, the Baltimore College of Dental Surgery, in 1840. In removing the proprietary and apprentice nature from dental education and patient treatment, the profession assumed the responsibility for setting *standards* for dental health, for diagnosis and treatment of oral diseases and disorders, and for assuring steady progress toward the elimination of disease through research and clinical patient care. Advances in technology (high speed, finger touch drills) and the use of local anesthetics have done much to reduce the anxiety and fear related to a visit to the dentist. We have gone from a drill-fill and denture-oriented profession to a prevention oriented profession during the past twenty years. Still, the overall health status of the people in this country has not changed significantly nor accordingly except in fluoridated communities where dental caries have been significantly reduced. I do not think that this should surprise us when we learn that only one third of the nation's children receive proper vaccination against diphtheria, pertussis, tetanus and polio even though these vaccines are available and free at public health clinics throughout the nation! This consumer apathy is similar to that found in the United Kingdom.

If we consider that half of the nation does not visit a dentist annually—for even a screening examination—then we have nearly a 50% needy nation! Not that they will all require treatment, but they do

need an oral health evaluation. In an effort to meet the dental health needs of a needy nation and anticipated demands for dental health services, our efforts have focused in the following areas during the past ten years:

1. *Modifications in dental education with the objective of increased dental health manpower.* Through Capitation awards to dental schools, we have been able to more than double the dental student enrollment within the past ten years. Schools responded to the Carnegie Commission's Challenge in the 1960's to increase enrollment to offset the anticipated increased demand for dental treatment. During the period 1971-1976 alone, there was a 41.3% increase in the numbers of dental graduates going from 3,775 in 1971 to 5,336 in 1976. During the same period of time, postgraduate dental first year enrollment showed a 2.6% decline going from 1,203 in 1971 to 1,171 in 1976. Dentistry has not been plagued with the over-specialization problem seen in medicine since less than 20% of present dental graduates enter specialty training (1976 graduates—5,336; 1976 postgraduate first year enrollment—1,171). Undergraduate enrollment was at 21,000 in 1976 whereas postgraduate enrollment was 2,718.

2. *Experimentation with expanded function auxiliaries.* This concept envisioned the utilization of highly skilled dental assistants and hygienists trained in dental procedures traditionally performed by the dentist that could be delegated without a reduction in the quality of service

received by the patient. Some 28 dental schools now receive TEAM grant funds from HEW to implement these programs. Also, it was envisioned that the expanded function auxiliary could be assigned along with dentists to rural and other underserved areas. The resolution passed by the American Dental Association in October, 1975* opposed the preparation of teeth and injection of local anesthetics by dental auxiliaries. However, TEAM grants continue to be funded in dental schools where the function of the auxiliary is not in conflict with the state dental practice act. The new Manpower Act continues to support program development in this area.

3. *Other experimentation involving private practitioners,* in the form of group and corporate practices, neighborhood health centers, HMO's, four-handed concepts, improved management techniques and increased utilization of auxiliary personnel, has taken place. All of these changes have helped improve the system of delivery but have in no way reduced the cost of dental services. In fact, during the nine years since the 1967 base line of 100.0 was established for the Consumer Price Index, the costs of operating a dental practice have increased more than 133% while dental fees have increased 66.5%. Our technological advances and our system of delivery to date have not demonstrated that we can reduce operating costs or that a saving can be realized by the consumer—the patient.

4. *Targeted research objectives.* At the present time and because of

* Resolution 861, American Dental Association, House of Delegates, 10/75.

limited research funding, federally directed research dollars have been directed toward studies that are of major incidence and concern. For dentistry, NIDR has provided substantial support for caries prevention studies, pain and anxiety control, for periodontal disease research, for behavioral research and for growth and development studies.

5. *Preventive dentistry* has become an accepted philosophy and ideal not just a movement. Every dental teaching institution now includes prevention in its curriculum. Studies on prevention conclude that constant reinforcement is essential and the most effective programs (outside of the military services) in this country have been those that included the homemaker or "mother figure" as the propelling stimulus. Recent data from Sweden (Ahlberg Study) demonstrate that effective continuity can be created in a preventive program that includes prophylaxis and fluoride applications performed by paraprofessionals on an annual basis. We have not done nearly enough in our preventive public education and media utilization. Maternal and Child Care Benefits include an Early Diagnosis, Prevention and Screening Program which provides for a dental component that is *not* being implemented to the extent that the federal funding authorization allows.

6. *General practice and total patient care concepts*: General practice residencies have increased and general dentistry clinical experiences are being added to dental curricula across the country. In 1976-77, there were 790 dentists in general practice residencies. The first year enrollment in general practice residencies has risen from 516 in 1971 to 733 in 1976. The dental graduate

of these programs is expected to be a highly skilled community oriented, general dentist and an expert diagnostician. He is expected to be the primary care provider for dentistry, to reduce the need for increased specialists (who come at a higher cost and perform limited services) and to be concerned with the general health and well being of the patient—including screening examinations for diseases such as hypertension, sickle cell disease, oral and pharyngeal cancer, diabetes, etc.

7. *Health Service Corps* assignments have become a reality during this period. In 1977, ninety-eight dentists had been placed in areas designated as shortage areas by the U.S.P.H.S. in an effort to correct the maldistribution problem.

All of these efforts to provide health care, where, when and for whom health services are needed have been less than adequate for we have not made the consumer an active participant in health—he has remained the health critic, the recipient and demander of health treatment. We have failed to properly educate the American public in self-assessment and health orientation. We have essentially left the consumer out of the *diagnosis* of health. The World Health Organization (WHO) has defined: "Health is a state of complete physical, mental and social well being and not merely the absence of disease or infirmity".¹¹ This is a holistic view of health but the WHO definition seems to imply that the patient has a role in the diagnosis of health in that his or her feeling of "well being" is the crucial criterion. This obviously cannot be the basis for a judgment of health—consider the "healthy" individual with oral cancer—undiag-

nosed. Does the patient become unhealthy only when he or she feels bad or when the cancer is detected following an oral examination and biopsy? The patient's feeling of well being is not a satisfactory guide to oral health status, but is a very good indicator of how he (she) priority-ranks health and what he will be willing to *give up* or *to do* to maintain a level of health that is acceptable. I would like to expand this concept further since our prevention studies clearly indicate the very significant role that the homemaker (mother or mother substitute) has in setting the level of quality for family health and nutrition. Can we afford to leave this type of responsibility to hostile, uninformed teenage parents with low levels of self-image and with unstable family structures?

The working mother and head of a family who must work eight hours per day, return home and cook and clean for her family with nothing better to look forward to than a repeat of this *day after day* and *year after year*, must receive substantial support and motivation if that family is to grow and reap the benefits of our affluent society. In my experiences in two inner city hospital clinics and in the dental school clinic, I have found that all of the needy mothers, regardless of race, want more out of life for their children than they have received. All of them are willing to bring their children in for treatment when *they* themselves are truly dentally indigent but they want their children to be secure and to have the things that other children have and things that they themselves were denied as children. They want a better *quality* of life for their children.

As we assess our present ability

or lack of ability to provide adequate dental health services for all people and look to the future, we must take very serious cognizance of plaguing questions such as what will be a more effective system of delivery than that which we now have? Who will be the dental health care providers and who will pay for dental care for people that cannot afford to pay? Can we realistically afford equitable health care for all? To this, I would answer a qualified *no*—under our present system of delivery and with our present attitudes toward health and nutrition. I predict, however, that once we have distilled the biases against federally funded health programs, such as Medicare, eliminated the abuses of such programs and instituted proper checks and balances, the Medicare type program can be written into a national health insurance system, partially subsidized by the federal government but controlled and managed by the private sector at state and local levels. This does mean that the federal funding must be distributed differently with more dollars going directly to health care with a base-option health program available to all Americans of all ages and various socio-economic strata.

As we anticipate and plan for the future, I believe the following strategies should be fully utilized in the U.S. and should be considered by developing countries as rational approaches to an improvement in the quality of life and in health care delivery:

1. *Health Planning and Resources Development.* In the U.S.A. this would mean full implementation of the Act passed in 1974 which provides for networks of Health Systems Agencies across the United States

for planning and development for regionally identified population groups known as Health Service Areas (204). The HSA's have been designated to serve population areas of 500,000 to 3 million people. The HSA, if properly implemented, will allow for proper utilization of health resources on a regional basis.

2. *Health Manpower Training.*

Federal support for health manpower training must continue, to assure that the quality of medical and dental education is maintained. Also, funding for incentives to practice in underserved areas may prove to be an effective mechanism for solving the manpower distribution problem. Scholarships and loans to students are essential if children of poor and moderate income parents are to be recruited to careers in dentistry and medicine. General dentistry and pedodontic residency training will increase based on provisions in the new Manpower Act of 1976.

3. *Significant Behavioral Modification.* This is needed, especially as it relates to personal attitudes toward health and nutrition. This goal will not be easy in the U.S. where alcoholism and drug abuse are still on the rise. In spite of the broad exposure given the Surgeon General's warning on cigarette smoking, there has been a rapid rise in smoking among women and teenagers. We have become flabby, comfortable "adults" with our carbohydrate-rich snacks, soft diets and lack of meaningful exercise. Air pollution continues to be a menace and viruses continue to be a major health problem. Still, we must begin somewhere and for impact and effect, I suggest that health education be included at all levels in the public school curricula. Churches and other

religious and civic organizations should include health education and counseling programs. A broader utilization of television and other communications media is needed that will allow prime time for health and nutritionally related subjects and sponsored at times where *families* can participate. Preventive dentistry and plaque control instruction are special areas that should be taken to the pre-school instructional level. Area Health Education (AHEC) Proposals will be used to establish community related networks for health education and communication.

4. *Fluoridation.* Where fluoridation is not available in the drinking water, special dental health programs are needed to assure that individuals receive the proper counseling and guidance regarding resources for fluoride, plaque control and diet. I had nearly forgotten what children's teeth looked like prior to mass fluoridation until I visited the state of Florida recently. The teeth of children from middle and upper income families looked far worse than the teeth from poverty stricken children in Washington, D.C. which has been a fluoridated community since the early 1950's.

5. *Auxiliary Utilization and Systems of Delivery.* Systems of delivery and utilization of auxiliary personnel must be changed to meet anticipated needs and demands for dental treatment. The availability and logistics of dental services do present problems for inner city parents who both work. Practices in centers and shopping malls that are well lighted and where parking and protection are already available could provide for evening dental services. Also, neighborhood dental clinics should extend

full services on weekends. Dental screening examinations could take place in the schools, churches and in mobile units that could be stationed in various parts of the country on an announced-date basis. Auxiliary personnel such as neighborhood health advisors, expanded function auxiliaries, nutritional advisors, etc. could be utilized in group practice settings and in health clinics to improve the quality of services rendered, to expand patient education and to increase the patient load.

6. *Distribution of Health Manpower.* A careful assessment of the maldistribution problem must be made and evaluated in terms of anticipated systems of delivery and need assessment. Experimental programs such as the Shortage Area Projects (SAS) that are currently being funded under the Special Projects Grants Program in the U.S. have exciting possibilities. The students in these programs spend time in remote sites (in a practicing dentist's office). Students are allowed to select their training site and the practitioner is considered off-campus faculty. The practitioner is sensitized to his responsibility as a teacher and recruiter through seminars sponsored by the school for off-campus faculty. Recruiting dental students from designated shortage areas who plan to return home to practice is another possible mechanism, but that will not provide immediate results. Still, it should be explored in the long range planning for manpower and manpower distribution in needy areas.

7. *Special Patient Care.* The dental student during his undergraduate dental training experience must be sensitized to the needs and peculiarities of special patients and on how to

treat these patients. Dental school clinic patients, in general, are moderate income paying patients. They are sensitized to oral health to the point that they come to *seek treatment* and they make visits on a regular basis because they know if they do not, they will be dropped or the student's progress will be affected. Our graduates need to know how to treat the fearful patients, the chronically ill patient, the developmentally disabled patient, the drug addicted patient. Therefore, we must make use of off-campus training sites such as neighborhood clinics, neighborhood health centers, hospitals, nursing homes and private offices to provide the clinical experiences that cannot be gained in a dental school clinic environment.

8. *Dental Research.* Research efforts should be directed toward the elimination of major disease categories such as: dental caries, periodontal disease, pain and anxiety control. Research results must be more effectively communicated to the practitioner and to the consumer especially as they impact on modification of methods of diagnosis and patient treatment. Also, educational research and research regarding systems of delivery and behavioral aspects of dental care are needed for efficiency and quality assessment purposes.

9. *Dental Education.* The dental educational institutions will continue to carry the ball for the integration of education, research and service aspects of the profession. The challenges that must be met focus on the role model of the future dentist and curriculum designs that will help prepare the dental practitioner of the future. Our struggle against *denturism* will be offset by the changing role of the dental practitioner—as

his (her) role in the diagnosis and treatment of oral disease, knowledge of interrelationships with systemic disease, and expertise in the utilization of auxiliary personnel are clarified, magnified and improved. Continuing educational models must be improved within the current academic structure as essential to the functioning of PSRO and recertification requirements of the future. Faculty recruitment, retention and development (especially for career teachers) will be areas of focus to assure critical mass faculty and a high level of quality for the academic base of the profession.

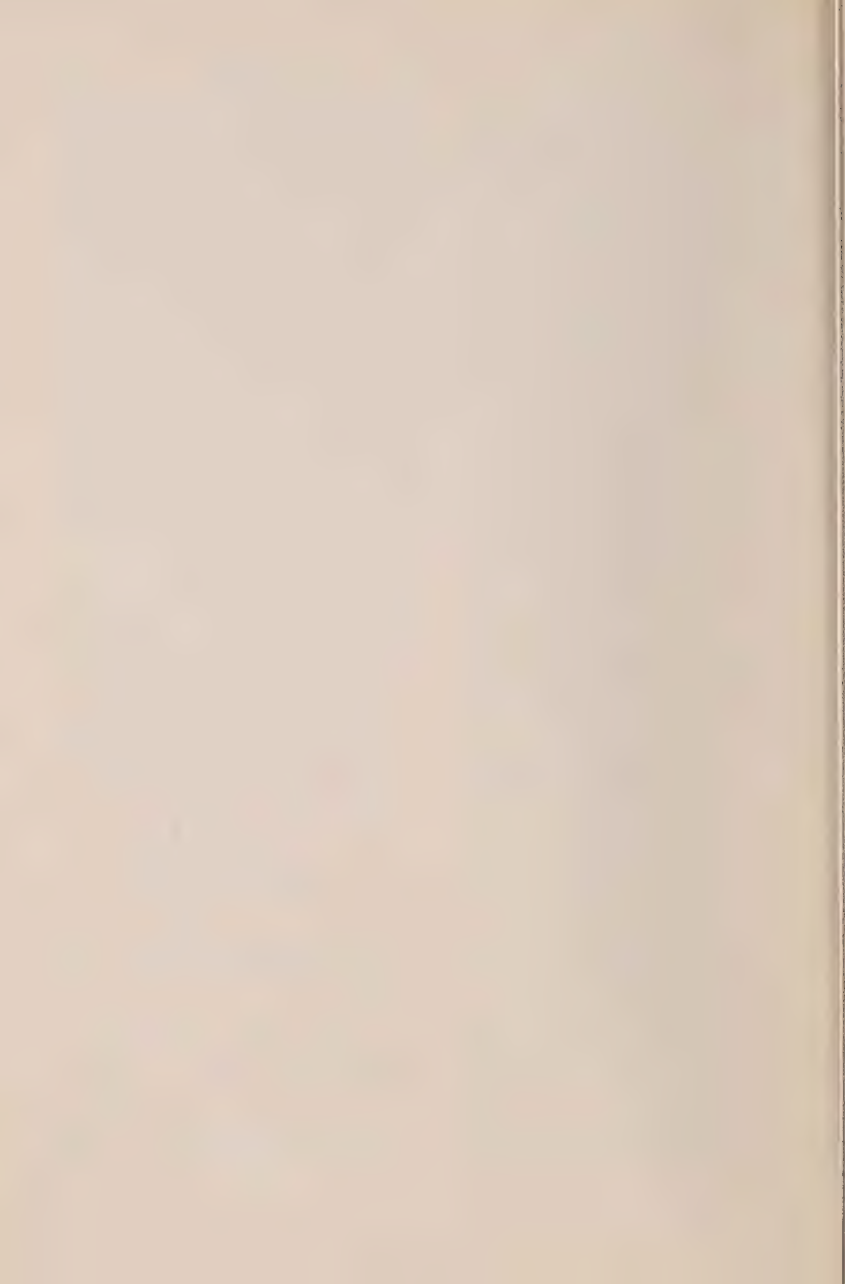
In summary, behavioral and attitudinal changes toward health must accompany our health planning and educational strategies for the future.

While we focus on quality and availability of oral health care and prevention of disease, if we are to improve the overall status of health and the quality of life, we must reduce crime, poverty, pollution, cultural restrictions and debilitating diseases that cripple the minds and bodies of our people. It is not enough for us to prolong life and eradicate disease. We must improve the quality of life for all people. In order to achieve this goal, health professionals must be *motivated* and *unified* in their ideals and purposes. Our effectiveness in the *achievement* of this mission will be measured by the *quality* and *status* of health in our children and in future generations to come.

Thank you.

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Oral Fusobacterium, Leptotrichia and Bacterionema:

I. Historical Survey and Taxonomic Considerations

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ABSTRACT

A review of the literature concerning the taxonomy of the genera *Fusobacterium*, *Leptotrichia* and *Bacterionema* is presented with emphasis on the factors which led to the confusion between these groups of bacteria.

These genera are presently easily differentiated from one another, although their taxonomic positions have not been resolved.

Recent DNA analysis data indicate that the present classification of *Fusobacterium* and *Leptotrichia* into the family *Bacteroidaceae* is not justified. The classification of *Bacterionema* with the *Actinomycetaceae* is likewise incorrect.

The precise mechanisms of oral bacterial disease in man remain unclear. Much of the problem of

studying these diseases lies in the complex nature of the oral microbiota, and the resulting difficulty of identification and classification. A large percentage of the total oral flora has not yet been isolated on laboratory media. Of those bacteria that have been isolated, many proved to be fastidious in their nutritional requirements and are difficult to maintain under laboratory conditions. To complicate matters further, some of the oral bacteria, especially the filamentous forms, show extreme cellular polymorphism.

Until very recently, bacterial taxonomy relied primarily on morphologic appearance, the Gram stain and occasionally simple biochemical tests for differentiation. This has resulted in an unreliable classification scheme for oral bacteriology. All too often, the morphologic overlap, Gram variability and fastidious nature of many oral bacteria led to erroneous identifications and con-

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siderable confusion in the literature.

To study the complex ecosystems and disease processes of the oral cavity, it is vital to have a reliable taxonomic system. Only after bacteria are adequately identified and classified can rapid progress be made in understanding oral bacterial diseases.

The genera *Leptotrichia*, *Fusobacterium* and *Bacterionema* are among those bacteria which have been subject to major taxonomic errors. Until recently these potentially pathogenic bacteria have been confused with each other in the literature.

This paper will report a survey of the literature concerning the taxonomy of these bacteria, emphasizing sources of earlier confusion and present criteria for differentiation.

HISTORICAL SURVEY

Fusobacterium

The bacteria presently known as *Fusobacterium* may have been described by Leeuwenhoek in 1683 (Dobell 1932). Two hundred years passed before attention was again focused on this anaerobic spindle-shaped microorganism when Plaut (1894) and Vincent (1896) observed fusiform bacteria in Vincent's angina and necrotizing ulcerative gingivitis (NUG).

The actual study of *Fusobacterium* was not possible until the organism was isolated in pure culture by Veillon and Zuber (1898). They were the first to give an adequate description of the morphology and some of the metabolic properties of the bacteria they called *Bacillus fusiformis*. *B. fusiformis* was shown to be a strict anaerobe that produced

a "fetid odor". This latter characteristic, caused by the production of butyric acid, is an important identifying feature of *Fusobacterium*. Unfortunately this characteristic was disregarded by many later workers.

In the early years of this century, Tunncliffe (1906) studied the polymorphic nature of *B. fusiformis*. Depending on the age and growth conditions of the culture, the organism was observed to exhibit all of the following morphologic characteristics: typical fusiform bacilli, long filaments, wavy forms resembling spirochetes, short bacillary forms, and spherical L-Forms. In fact, all of these forms were, at times, found to exist in the same culture. At the time that she began her work, it was not possible to isolate and grow spirochetes in pure culture. This, in addition to the presence of wavy forms of *B. fusiformis*, led her into a twenty year study to show that *B. fusiformis* was only a stage in the life cycle of oral spirochetes (Tunncliffe 1923). Her work was not widely accepted, probably because the "spirilla" in the pure *B. fusiformis* cultures were non-motile, unlike the spirochetes found in vivo. In her later work, she claimed to have demonstrated motile "spirilla" in the pure cultures, complete with demonstrable flagella (Tunncliffe 1923). However, her work was not reproducible (Pratt 1928).

A great number of strains of *B. fusiformis* were isolated early in the twentieth century. Krumbwiede and Pratt (1913) attempted to devise the first classification system of *B. fusiformis*. They isolated 15 strains from plaque, noma and "gangrene" (probably NUG), and were unable to find any consistent morphological differences among them. Their fer-

mentation studies revealed that the only differential characteristic was the ability of some strains to utilize sucrose while others could not.

Knorr (1922, 1923) proposed the generic name *Fusobacterium* for these bacteria and used morphologic criteria to divide the old species *Bacillus fusiformis* into three new species called *F. polymorphum*, *F. nucleatum* and *F. plauti-vincenti*. Although Knorr based his classification system on only four isolates, it was used until very recently with only one slight alteration, the substitution of the species name *F. fusiforme* for *F. plauti-vincenti*. Subsequently, all oral *Fusobacterium* have been combined into the species *F. nucleatum*.

Workers began to realize that morphology alone was not an adequate characteristic for classification of the *Fusobacterium*. Varney (1927) attempted a serological classification scheme and proposed four types of *Fusobacterium* on the basis of agglutination tests. However, none of his isolates had an "unpleasant odor", opening for question the true identity of the strains he used.

Other classification schemes were devised over the next several years using various criteria for differentiating species of *Fusobacterium*. The classification system presented by Slanetz and Rettger (1933) was based primarily upon colonial morphology. For isolation, they devised the first selective media for *Fusobacterium*, incorporating potato extract agar with gentian violet.

It became apparent to some investigators that they were confusing two distinct but morphologically similar bacteria with each other; the organisms presently placed into the

genus *Leptotrichia*, with those of the genus *Fusobacterium*. Spaulding and Rettger (1937) classified their isolates of *Fusobacterium* into two groups. In the first group, they placed those strains that were weakly sacchrolytic, produced hydrogen sulfide, were indole positive, produced a foul odor, and fermented glucose, levulose, and sometimes sucrose. The second group, which was clearly distinct from the first, consisted of those strains that were indole negative and did not produce hydrogen sulfide. In addition, they were strongly sacchrolytic, able to produce much more acid per unit time than the first group, and were able to ferment maltose and trehalose in addition to glucose levulose. A foul odor was not produced by these strains. They tried to further categorize the isolates by serological tests (agglutination), but found too much interference by autoagglutination characteristics of *Fusobacterium*. Although Spaulding and Rettger did not specify the identity of their second group of isolates, they did realize that it was quite different from their first group. Their second group resembled what is currently considered *Leptotrichia buccalis*.

During the same year that Spaulding and Rettger published their work, Hine and Berry (1937) did a morphologic study of *Fusobacterium* that paralleled Knorr's earlier work, using 104 isolates. This study became the basis for the *Fusobacterium* section of the 7th edition of Bergey's Manual (Hoffman 1957). Since there were no accepted taxonomic guidelines separating *Fusobacterium* from *Leptotrichia*, it is not surprising that Hine and Berry did not recognize that they were working with both of these bacteria. Their species *F. dentium*, supposedly corresponding

to Knorr's *F. plauti-vincenti*, has the characteristics of *L. buccalis*.

Shortly afterwards, Boe (1941) proposed separating these two distinct groups of bacteria into two genera. The weakly sacchrolytic, butyric acid-producing organisms were placed into the genus *Fusobacterium*. Since there were no known distinguishing biochemical differences between the oral isolates of *Fusobacterium*, and all strains seemed to be too polymorphic for morphological differentiation, he suggested grouping all isolates into one species. The species was to be called *F. plauti-vincenti*. This designation should be distinguished from the interpretation of Knorr's *F. plauti-vincenti* as presented by Hine and Berry (1937) and later by Hoffman in the 7th edition of Bergey's Manual (Hoffman 1957). Hoffman's *F. plauti-vincenti* was strongly sacchrolytic, indole negative bacteria which was unable to produce butyric acid or hydrogen sulfide. Hoffman's *F. plauti-vincenti* is now recognized as being identical to the bacteria of the present genus *Leptotrichia*.

Much of the earlier confusion concerning the classification of these microorganisms has been carried on until very recently. The section on *Fusobacterium* in the 7th edition of Bergey's Manual, was based on earlier misconceptions. The designation *F. plauti-vincenti* was changed to *F. fusiforme*, and as stated above, Hoffman, who wrote the section, proceeded to describe *L. buccalis*. Fortunately, the observations of Boe defining differential characteristics of *Leptotrichia* and *Fusobacterium* have been reinforced by numerous studies including those of Jackins and Barker (1951), Omata and Braunberg (Omata and Braunberg

1960), Hadi and Russell (1968) and Werner, Neuhaus and Hussells (1971) and are now accepted by investigators working with these bacteria.

Subsequently, the 8th edition of Bergey's Manual included in the genus *Fusobacterium* only the butyrate positive strains that were first clearly defined by Boe (1941). All oral strains were placed into *F. nucleatum*, the type species of the genus. *F. fusiforme*, *F. polymorphum* and *F. nucleatum* are now considered to be synonymous (Buchanan and Gibbons 1974).

In addition, the genus *Fusobacterium* has been placed into the family *Bacteroidaceae*, using morphology and simple metabolic tests as the major criteria for classification (Buchanan and Gibbons, 1974).

Over the past two decades, methods have been developed which can give phylogenetic relatedness information, obviating the total dependence on phenotypic characteristics for classification. The advent of the computer made possible the application of numerical taxonomy. Numerical taxonomy eliminates the dependence on single traits, such as morphology and Gram stain for classification by equally weighing all known characteristics of a bacteria. Usually well over one hundred (100) traits are used for comparison. Bacteria are clustered according to overall trait similarity. Such a system is a vast improvement over the previous taxonomic methods, but, there are still shortcomings. The assumption is made that by studying as many phenotypic characteristics as possible, a reflection of the bacterial genotype can be gained. Unfortunately, similarity of phenotypic

traits does not necessarily accurately indicate genetic relatedness. Close similarity to may be due to parallel or convergent evolution in a similar environment. It tells little about the phylogenetic similarities of enzyme systems producing phenotypic traits. Numerical taxonomy can only give an indication of phylogeny.

At least two other methods presently available can give much more reliable phylogenetic relatedness data but are considerably more time consuming and can be difficult to interpret. These are DNA analysis (including mole percent of each nucleotide in DNA and nucleic acid homology studies) and protein homology studies. Both methods rely on either the nucleotide composition or sequence in DNA. The nucleotide content (mole percent G+C) is a determination of the proportion of each nucleotide in DNA, not their sequence. Similar proportions of nucleotides in the DNA of bacteria indicates that genetic relatedness is possible. However, it must be emphasized that such similarity in the proportion of nucleotides only means that sequence similarity is possible. The nucleotide sequence can be very dissimilar even if the relative quantity of each nucleotide (mole percent G+C) is the same. On the other hand, the more dissimilar two species of DNA are in nucleotide contents the less likely there is to be close sequence similarity.

One of the techniques used to compare nucleotide sequence similarity is nucleic acid hybridization. This technique ideally depends upon identical nucleotide sequences in DNA and can demonstrate genetic homology between bacteria within the same genus or in closely related genera. Nucleic acid hybridization

is usually not adequate to study similarity among distantly related bacteria.

Protein or enzyme homology studies use immunologic techniques to detect slight amino acid differences in conserved enzymes among bacteria. Like the nucleic acid homology studies, this technique yields data indicating nucleotide sequence homology. Furthermore, it can be used to compare the genetic homology among genetically diverse bacteria.

Page and Krywolap (1976) carried out mole percent G+C content determinations and nucleic acid hybridization experiments using two strains of *Fusobacterium* previously designated as *F. polymorphum* ATCC 10953 and *F. fusiforme* ATCC 23736 (Page and Krywolap 1976). While the mole percent G+C values for *Fusobacterium* strains tested were approximately 26%, those of other *Bacteroidaceae* reported were over 41%; a minimum difference of about 15%. It is therefore unlikely that *Fusobacterium* belongs in the same family as the *Bacteroidaceae* reported in the literature.

The nucleic acid hybridization data showed a 78% similarity between the DNA of the *Fusobacterium* strains tested (Page and Krywolap, 1976). Although there are no definite criteria separating species, there have been reports of bacteria accepted as belonging to different species having a greater similarity than this (Hoyer and McCullough 1968, Jain, Radsak and Manuheim 1974, Ritter and Gerloff 1966, Roof, Mundt and Riggsby 1974). Further investigation is indicated to make sure that the combining of all oral *Fusobacterium* into one species was not premature.

Leptotrichia and *Bacterionema*

As discussed above, *Leptotrichia buccalis* has been repeatedly confused with the *Fusobacterium*. On the basis of cellular morphology, *Leptotrichia* and *Fusobacterium* cannot be consistently distinguished from each other, although they can be separated on the basis of biochemical tests (Buchanan and Gibbons 1974, Werner, et al. 1971).

In contrast, the confusion between microorganisms presently referred to as *Leptotrichia* and *Bacterionema* is more difficult to understand. *L. buccalis*, the type species of the genus *Leptotrichia*, is a Gram negative non-branching anaerobe. *Bacterionema matruchotii*, the type species of the genus *Bacterionema*, is a Gram positive, very polymorphic facultative organism. It has a bacillary-like body attached to the end of filaments and occasionally shows dichotomous branching.

Such taxonomic errors were the result of early oral microbiologists' attempts to be consistent with papers published prior to 1900. These papers gave names to bacteria which were not well described. The bacteria were rarely successfully isolated and grown, and the work was based on the morphologic appearance of wet mounts and smears of oral debris and plaque (DeToni and Trevisan 1889, Robin 1853, Trevisan 1879 and Trevisan 1889). The bacteria presently designated as *B. matruchotii* and *L. buccalis* were both mistakenly designated *L. buccalis*.

As previously mentioned, Leeuwenhoek was the first to observe, describe and illustrate oral filamentous bacteria (Dobell 1932). Robin (Robin 1853) reported seeing filamentous bacteria in wet mounts of

tooth scrapings and made the following descriptions, as per Gilmour, Howell and Bibby (1961): "Genus *Leptothrix* Kutz. Filaments very thin, not ramified and not coherent. Species 14-*Leptothrix buccalis* Ch. R. With straight or curved rigid filaments, not moniliform, colorless, with blunt ends, adherent at the base in an amorphogranular stroma. Length .020 to .100mm, width .0005mm. Habitat. On the surface of the tongue, between the teeth, in the cavity of decayed teeth and in the juices of the stomach and intestine." Robin placed these microorganisms into the genus *Leptothrix* because he thought they were related to the aquatic algae. Although he is credited with being the first scientist to describe *L. buccalis*, we will never be certain of what bacteria he was observing. It was partially because of reliance on such descriptions that the identification of oral bacteria was so difficult.

The name *Leptothrix buccalis* became the catch-all genus of oral microbiology. This led Miller (1890) to make the following statement:

"*Leptothrix buccalis* is a name chosen by Robin for those organisms in the human mouth which were formerly described as animalicula, tooth-animalicules, Buhlmann's fibers, denticolae, etc. Almost every living organism in the mouth was designated by this common name . . . We must guard against the very common error of considering every thread-forming organism which occurs in the oral cavity, or is obtained in pure culture from the juices of the mouth, as *Leptothrix buccalis*, inasmuch as threads are formed by various microorganisms . . . The name *Leptothrix* designates no

particular organism possessing peculiar characteristics, and the name deserves to be retained as little as denticola, Buhlmann's fibers, etc.; the more so since it has always been the expression for an obscure and erroneous conception."

Miller then went on to make a proposal that only increased the problem: "For those bacteria growing in threads, whose biology is too little known to define their relation to other mouth bacteria, or to form a separate group with distinct characteristics, I propose the provisional name of *Leptothrix innominata*." Fortunately, this suggestion did not gain wide acceptance in the scientific community.

Before Miller's classic text on oral microbiology was published in 1890, Trevisan (1879) realized that oral filamentous bacteria were not related to aquatic algae. He suggested removing these bacteria from the genus *Leptothrix*, and placing them into a new genus to be called *Leptotrichia*, the type species being *L. buccalis*. Again, the description he gave was too vague to be very useful, and it differed from Robin's (Robin 1853) description. Trevisan probably was, however, describing what we now know as *Leptotrichia* or *Fusobacterium*. Unfortunately, Trevisan and DeToni made several very confusing modifications of Trevisan's genus *Leptotrichia*, including the formation of a new ill defined genus they called *Rusmussenia* (DeToni and Trevisan 1889, Trevisan 1889). The genus *Rusmussenia* supposedly consisted of some of the bacteria originally placed in the genus *Leptotrichia* by Trevisan. The type species was to be called *R. buccalis*.

Many years later Kligler (1915) isolated and grew a microorganism which he called *Leptothrix buccalis*, and described it as: "A thick long, straight, or curved thread with a club head at one extremity . . . They are anaerobic, facultative-aerobic, non-motile, non-branching threads." Kligler then went on to describe "branching forms". His description generally fits that of the organisms presently placed into the genus *Bacterionema*. The microorganism in the photomicrographs accompanying his article are almost certainly *Bacterionema*, complete with the unique bacillary-like bodies attached to the ends of filamentous cells. Kligler's paper is referred to in many more recent articles, and probably is the single most important reason for confusing microorganisms presently called *B. matruchotii* with the Trevisan-type microorganisms of the genus *Leptotrichia*.

Leptotrichia buccalis can grow under the same conditions as *Fusobacterium*. It is reasonable to assume that *Leptotrichia* could have been successfully isolated and grown in 1898, along with *Fusobacterium* (Veillon and Zuber 1898). However, Wherry and Oliver (1916) were first to isolate and grow what is now called *Leptotrichia*, and to recognize it as a separate genus. The photographs accompanying the Wherry and Oliver paper, the description and the lack of any mention of foul odor all indicate that they were probably working with *L. bucca* and not *Fusobacterium*. Unfortunately, the importance of this study was not appreciated for the next twenty years. Most significantly, the Winslow Committee (Winslow, et al. 1917) and Bergey's Manual

through the 5th edition (Breed 1948) depended upon the vague earlier papers for the basis of their descriptions.

The Winslow Committee was established in 1917 to prepare a revision of the earlier bacterial taxonomy systems, according to the International Rules of Botanical Nomenclature. The committee classified *Leptotrichia* as a member of the family *Mycobacteriaceae*, their description, aside from being inadequate, does not correspond to the descriptions of either Robin (1853), or Trevisan (1879). It is much closer to the Kligler (1915) description, but differs from it on several points. It is probably a composite of several works, including Kligler's.

One year later, *L. buccalis* was placed into the family *Actinomycetaceae* by Buchanan (1916). His work, as well as that of Wherry and Oliver (1916) were ignored in the first edition of Bergey's Manual (Bergey, et al. 1923) which continued the earlier confused description of this genus. According to Breed (1948) the type species description used in Bergey's Manual was based on Vignal's (1886) description of a spore-forming bacteria.

It was not until 1937 that the scientific community began to report definitive criteria for differentiating *L. buccalis* from the *Fusobacterium* (Spaulding and Rettger 1937). As was mentioned in the *Fusobacterium* section, Spaulding and Rettger realized that the bacteria which they called *F. plauti-vincenti* differed from the other *Fusobacterium* because they did not produce hydrogen sulfide, were indole negative, strongly saccharolytic, and most importantly, did not produce butyric acid as its major end product. These are now

the primary criteria for the differentiation of *Leptotrichia* from *Fusobacterium*.

About fifteen years ago, definitive criteria separating *Leptotrichia* from *Bacterionema* were clarified. According to Gilmour, et al. (1961) in 1935 Bibby (1935) was first to observe that the original Robin (1853) and Trevisan (1879) descriptions of *L. buccalis* were different from the description given in Bergen's Manual. Again, according to Gilmour, et al. (1961) in the late 1930's Thjotta, Hartman, and Boe also pointed out the difference between the microorganism called *L. buccalis* by Bergey's Manual, 5th edition, and the descriptions of Robin, Trevisan, and later Wherry and Oliver. Soon after, Boe and Thjotta (1944) finally clarified this genus, presenting a description which was the best up to that time.

The separation of what is presently called *B. matruchotii* from *L. buccalis* still was not universally accepted. Both the 6th and 7th editions of Bergey's Manual did not recognize the genus *Leptotrichia*. Richardson and Schmidt (1959) published a study showing the difference between the Kligler (1915) and the Trevisan (1879) and Thjotta (Boe and Thjotta 1944) organisms. They suggested that the Thjotta-type organism be placed into the family *Lactobacillaceae*, genus *Lactobacillus* or *Catenabacterium*. They also suggested placing the Kligler type organism into the family *Actinomycetaceae*, genus *Nocardia*. They noted that the latter classification was not satisfactory, but it was the best that could be done with their knowledge of the Kligler type bacteria. The Kligler bacteria differs from true *Nocardia* in its morphology and facultative

character. A similar observation was made by Howell and Rogosa (1958) one year earlier.

Davis and Baird-Parker (1959) suggested calling the Kligler bacteria *Leptotrichia dentium*. The Thjotta type microorganism was to retain the designation of *L. buccalis*. In spite of the great differences between these two types of organisms, they wished to keep them in the same genus.

The most definitive work on the bacteria known as *Leptotrichia* was done by Gilmour and her co-workers (Gilmour 1961, Gilmour and Beck 1961, Gilmour, et al. 1961). In addition to presenting an excellent historical background and a study of growth and biochemical characteristics of these bacteria, they proposed separating *L. buccalis* and *L. dentium* into two separate genera. The Kligler-type bacteria would be placed into a new genus to be called *Bacterionema*. The Trevisan type bacteria, as described by Wherry and Oliver (1916), and Boe and Thjotta (1944), and now referred to as the Thjotta organism, was to remain in the genus *Leptotrichia*. It was suggested that *L. buccalis* be placed "in the family *Lactobacillaceae*, tribe *Lactobacilleae*," and *B. matruchotii* should be placed into the family *Actinomycetaceae*.

Precisely where *L. buccalis* and *B. matruchotii* should be taxonomically placed in relation to other oral bacteria has not yet been resolved.

Melville (1965) studied several *Actinomycetes* including *B. matruchotii* using numerical taxonomy and concluded that it was definitely separate from *Nocardia* and *Actinomyces*, and may be closer to the *Corynebacteria*. A similar conclusion

was reached by Snyder, et al. (1967) on the basis of serological studies using cell wall antigens. These conclusions are supported by mole percent G+C data reported by Page and Krywolap (1974). The average mole percent G+C content of *B. matruchotii* DNA is 54. That of other recognized members of the *Actinomycetaceae* were reported to be greater than 62%, while *Corynebacteria* are about 56%. These data show *B. matruchotii* is much more likely to be closely related to the *Corynebacteria* than the *Actinomycetaceae*. In the 8th edition of Bergey's Manual, *B. matruchotii* was recognized as a valid species, but still is classified in the family *Actinomycetaceae* (Buchanan and Gibbons 1974). A streptococcal variant of *B. matruchotii* has been reported in the literature (Ennever, Streckfuss and Takazoe 1973, Streckfuss and Smith 1970). The mole percent G+C data (Page and Krywolap 1974) indicates that this "variant" is not closely related to *B. matruchotii* and is probably a streptococcal contaminant.

The suggestion by Gilmour, et al. (1961) that *L. buccalis* be placed into the family *Lactobacillaceae* has also been challenged. Hofstad, Kristofersen and Selvig (1972) carried out a study of the cell wall of *L. buccalis* on the electron microscope, and determined that it possessed a typical Gram negative cell wall. They concluded that it could not be a member of the *Lactobacillaceae*, and suggested placing it within the family *Bacteroidaceae*, where in fact, it has been placed in the 8th edition of Bergey's Manual, along with the *Fusobacterium* (Buchanan and Gibbons 1974). However, the mole percent G+C values (Page and Krywolap 1976)

indicate that *L. buccalis* DNA has a value similar to that of the *Fusobacterium* and well below that of other *Bacteroidaceae*. In addition, the similar unusually low mole percent G+C values for these two genera could indicate a close phylogenetic relationship. Nucleic acid hybridization between *Fusobacterium* and *Leptotrichia* strains showed less than thirty percent homology, which is not conclusive evidence supporting or detracting from this possibility (Page and Krywolap 1976). Hawley and Falkler (1975), however, showed no immunologic cross re-activity between crude antigens of *L. buccalis* and *Fusobacterium* species tested, which may indicate a low probability of a close phylogenetic relationship.

SUMMARY

The precise taxonomic position of *Fusobacterium*, *Leptotrichia* and *Bacterionema* has not been resolved but

the descriptive literature is now adequate enough to prevent further confusion between these distinct groups of bacteria.

Although *Fusobacterium* and *Leptotrichia* have been placed into the family *Bacteroidaceae* there is evidence to show this placement to be incorrect. Furthermore, DNA-DNA hybridization data on a very limited scale indicates that the combining of all oral *Fusobacterium* into one species may have been premature.

The Gram positive *Bacterionema* are distinct from the Gram negative filamentous bacteria. Although *Bacterionema* has been placed into the family *Actinomycetaceae* the published mole percent G+C values for this genus indicates that it belongs elsewhere.

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**Oral Fusobacterium, Leptotrichia
and Bacterionema:**

II. Pathogenicity: A Review of the Literature

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ABSTRACT:

A review of the literature concerning the pathogenicity of *Fusobacterium* species, *Leptotrichia buccalis* and *Bacterionema matruchotii* is presented. *Fusobacterium* has long been associated with gingivitis, periodontitis, necrotizing ulcerative gingivitis (NUG) and Vincent's angina. Circumstantial evidence, such as serological data and increase in cell numbers with inflammation, point toward an active role in these disease processes. Evidence of similar nature also indicates a possible role for *L. buccalis* in gingivitis, periodontitis and NUG. *B. matruchotii* is one of a number of calculus forming bacteria. By contributing to calculus formation, it probably plays a secondary role in periodontal disease.

Gram-negative filamentous bacteria including those of the genera *Fusobacterium* and *Leptotrichia* have been associated with gingivitis, peri-

odontitis, necrotizing ulcerative gingivitis (NUG), and its extension, Vincent's angina and noma by numerous authors over the last century. They were consistently found in oral disease processes along with other morphologic types of microorganisms.

In spite of such associations, a cause and effect relationship has never been demonstrated. This is probably because of the complex ecologic nature of these diseases in addition to the problems of adequate isolation and accurately identification of oral bacteria.

Bacterionema matruchotii has been consistently confused with and mistakenly called *Leptotrichia*. Unlike the Gram-negative *Fusobacterium* and *Leptotrichia*, the Gram-positive bacteria of the genus *Bacterionema* have not been implicated as direct participants in the previously mentioned periodontal diseases.

It is the objective of this paper to

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present a selective review of the literature concerning the pathogenicity of these three bacterial genera in oral disease.

FUSOBACTERIUM:

Miller is credited as being the first modern scientist to describe fusiform bacteria associated with the disease now called NUG. (Burnett & Scherp 1968). However, no reference to the involvement of fusiform bacteria could be found in his book although the disease was mentioned several times (Miller 1890). Soon after, Babes (1893) definitely described fusiform bacteria associated with NUG, but failed to follow through on his observation with additional research.

Plaut (1894) described fusiform bacteria as being associated with spirochetes in a disease presently known as Vincent's angina. Vincent (1896) made a study of 47 cases of "hospital gangrene" (Vincent's angina and NUG) and found fusiform bacteria in association with spirochetes in 40 of these patients. From that time, spirochetes and fusiform bacteria were assumed to be the etiological agents of the foul-smelling destructive lesions of the oral region characteristic of what we now call Vincent's angina and NUG.

Veillon and Zuber (1898) were the first to produce clinical disease in rabbits and guinea pigs, by injecting quantities of *B. fusiformis* (*Fusobacterium*) in pure culture. They found that an abscess was produced at the injection site, but healed quickly and produced no severe symptoms. The lesion was not the typical necrotic lesion found in human fusospirochetal infections.

Having been so commonly isolated from necrotic lesions, many believed that *B. fusiformis* was a true pathogen. However, it was later found that it is part of the normal flora and must be an "opportunistic" pathogen (Muhlen & Hartmann 1906). It has been estimated that *Fusobacterium* makes up approximately 3% of the total cultivable facultative and anaerobic microorganisms found in debris from the gingival sulcus (Gibbons et al. 1963). There is some conflict in the literature as to whether there is an increase in the number of *Fusobacterium* during gingivitis, periodontitis and NUG. Gibbons et al. (Gibbons et al. 1963) were not able to determine a significant difference in the number of *Fusobacterium* between normal and periodontally involved patients, using gingival debris as their source material for bacterial counts.

In another study, utilizing a larger number of patients, Hadi and Russell (1968) did find a significant rise in the number of *Fusobacterium* in the saliva of people with NUG. Their mean *Fusobacterium* count in the control group was 2.7×10^5 /ml saliva, as compared to 1.8×10^6 /ml saliva in the NUG group. They also reported a significant rise in the number of *Leptotrichia* during NUG. Other studies by Krygier et al. (1973) tend to support the work of Hadi and Russell.

More recently van Palenstein Helderma (1975) sampled a group of patients scored according to the gingival index of Löe and Silness (1963) for differential counts of several types of bacteria. He was able to show that *Fusobacterium* levels increase with the index scores. He collected his

samples on sterile paper points from periodontal pockets and gingival crevices after the removal of supragingival plaque. Bacteria were grown on non-selective (non-inhibitory) media. His samples most likely included microorganisms from the superficial layer of subgingival plaque as well as gingival debris. Sabiston and Gold (1974) emphasized the common involvement of *Fusobacterium* in severe oral infections by showing it to be involved in seven out of eight oral abscesses studied. In five of these lesions *Fusobacterium* was considered the major bacterial constituent.

Similar observations were reported by Slots (1977). In his study of the predominant cultivable microflora of advanced periodontitis in eight patients, aged 34-48, *Bacteroides melaninogenicus* and *Fusobacterium nucleatum* constituted the majority of the isolates; however, their mutual proportions differed considerably between the samples.

Syed et al. (1977) in a recent bacteriological study of periodontitis associated plaque of beagle dogs reported *Fusobacterium nucleatum* to be present in higher proportions in the subgingival plaque as compared to supragingival plaque.

Fusobacterium are capable of utilizing amino acids as their sole energy source (Barker 1961). In addition, glucose utilization can be carried out at the same time with no repression exerted on amino acid catabolic enzymes (Loesche & Gibbons 1968). These features theoretically make the *Fusobacterium* an ideal organism for growing in an anaerobic environment where large quantities of protein are present, such as in the periodontal pocket. A

very important potential pathogenic characteristic of *Fusobacterium* is the presence of biologically active endotoxin (Boe 1941, Hofstad, Kristoffersen & Selvig 1972, Kristoffersen & Hofstad 1970a, Kristoffersen & Hofstad 1970b). However, in spite of the circumstantial evidence mentioned, no cause and effect relationship has been shown implicating *Fusobacterium* as a cause of human periodontal disease. The type of study of Veillon and Zuber (1898) on the pathogenicity of *Fusobacterium* has been repeated many times over the years, and the general conclusion is that *Fusobacterium* in pure cultures is not capable of causing the typical foul-smelling "fusospirochetal" lesion. However, none of the studies used humans as subjects, with the exception of Boe's self-inflicted infection which will be discussed later.

Kritchewski and Seguin (1920) were the first to produce a "typical fusospirochetal lesion" in guinea pigs, similar to those seen in man. They used a combination of *Fusobacterium*, spirochetes, and either a "greening streptococcus" or *Staphylococcus albus*, noting that none of these bacteria alone could produce the lesion. This was the first evidence demonstrating the synergistic characteristic of the bacteria in fusospirochetal lesions.

The ability of *Fusobacterium* in pure culture to cause severe infection in man was demonstrated by Boe. According to Burnett and Scherp (1968), Boe injected his forearm with 0.4 ml of pure culture of *Fusobacterium*, originally isolated from a human brain abscess. After six days, an abscess formed with severe edema of the arm, along with enlargement of the axillary lymph

nodes. Surgical intervention was necessary to contain the infection. Although a typical fusospirochetal type lesion was not produced, this type of microorganism alone certainly is capable of producing disease. This, in addition to the recent report of Sabiston and Gold (1974) already discussed, indicate that under certain conditions *Fusobacterium* can cause severe acute infections in man.

The experimental results of Kritchewski and Seguin are reproducible, and investigators believe that a combination of bacteria is needed to produce the usual type of lesions seen in NUG, Vincent's angina, and noma (Burnett & Scherp 1968, MacDonald, Sutton & Knoll 1954). Most attempts at bacterial isolation from lesions observed in these ulcerative diseases have yielded *Fusobacterium*, various spirochetes and several other types of bacteria. MacDonald et al. (1954) isolated 17 different type of bacteria from a fusospirochetal infection of a guinea pig. They determined that a minimum of 4 types of bacteria were necessary to produce the typical fusospirochetal lesion. The essential organisms included 2 strains of *Bacteroides*, one motile gram-negative rod and a facultative diphtheroid. Some of these organisms could be replaced by *Fusobacterium* and spirochetes, although it was demonstrated that the guinea pig fusospirochetal type of lesion could be produced without *Fusobacterium* or spirochetes. The authors concluded that the so-called fusospirochetal lesion is bacteriologically non-specific. It seems that the lesion is a complex ecosystem; each ecological niche probably can be filled by any of a number of different bacteria,

rather than needing one specific microorganism.

There is immunologic evidence to show that *Fusobacterium* usually does play a part in the type of lesion seen in NUG, as well as common gingivitis and periodontitis in man (Hadi & Russell 1969, Kristoffersen & Hofstad 1970a, 1970b, Wilton, Ivanyi & Lehner 1971). Antibody against *Fusobacterium* has been demonstrated in a large percentage of the population, but there was no correlation observed between antibody titer and severity of periodontal disease (Kristoffersen & Hofstad 1970a). Wilton et al. (1971) obtained the same results using serum antibodies; however, they were able to correlate severity of periodontal disease to increased cellular immunity toward *Fusobacterium*. They measured a significant difference in lymphocyte transformation tests between patients with NUG and gingivitis, compared to a control group.

The precise role of *Fusobacterium* in disease of the oral cavity is still not known. We can only state that they are an important part of the oral flora and probably play an active role in disease processes.

LEPTOTRICHIA:

Evidence has implicated *L. buccalis* as possibly playing a major role in periodontal disease. Production of biologically active endotoxin by *L. buccalis* has been demonstrated by numerous workers (Araujo, Varah & Mergenhausen 1963, Gustafson & Kroeger 1962, Hofstad & Selvig 1969, Knox & Parker 1973, Mergenhausen 1960). Gustafson et al. (1966) compared the biologic activity of *L.*

buccalis endotoxin to that of *Escherichia coli* using LD₅₀, pyogenic activity, leukocytic response and the Schwartzman reaction in rabbits. They concluded that *L. buccalis* endotoxin is very potent. Mergenhagen, de Araujo and Varah (1965) used hemolysis tests with sheep red blood cells, coated with "crude" *L. buccalis* antigens, to show the presence of anti-*Leptotrichia* antibody in eight out of nine human sera tested. The same test, carried out using other gram-negative bacteria found in the oral cavity, showed a much lower frequency of what was called "significant antibody titers." In addition, the percentage of *L. buccalis* in the saliva of people with NUG has been reported to be three to four times that found in control groups (Hadi & Russell 1968). A numerical increase was not demonstrated in a similar study of subjects with periodontitis (Hadi & Russell 1969). Gilmour and Nisengard (1974) showed that antibody titer to *L. Luccalis* crude lysate antigens are usually present in periodontal disease.

More recently, Falkler and Hawley (1975) and Hawley & Falkler (1975) showed that 5 of 6 periodontitis patients tested had antibody to both protein and lipopolysaccharide components of *L. buccalis* cell walls using three different serological tests. They are presently working on a study to correlate titer differences with severity of periodontal disease.

As in the case of *Fusobacterium*, it is difficult to demonstrate a cause and effect relationship with *L. buccalis* and periodontal disease. Demonstration of differential antibody titers in disease processes is only circumstantial evidence.

BACTERIONEMA:

There is no evidence to date to show that *B. matruchotii* is directly involved in the common periodontal or oral bacterial diseases. Many studies have shown that *B. matruchotii* is capable of forming intracellular hydroxyapatite crystals in culture and probably is involved in calculus formation (Bulleid 1925, Ennever 1960, Ennever, Vogel & Heaker 1970, Ennever, Vogel & Takazoe 1968, Rizzo et al. 1962, Takazoe 1961, Takazoe & Ennever 1969, Takazoe, Kurahashi & Takumas 1963). Thus, it appears that *B. matruchotii* may play an indirect role in gingivitis and periodontitis by contributing to calculus formation.

SUMMARY:

Enough circumstantial evidence is available to indicate that *Fusobacterium* can play a role in gingivitis, periodontitis and NUG. However, such disease processes are probably due to synergistic bacterial infections with numerous eoniches. It seems that each of these niches can be occupied by any number of bacteria which have the necessary metabolic characteristics to successfully fill the niche. There is, however, evidence to show that *Fusobacterium* is frequently the major bacteria involved in oral abscess formation.

Likewise, similar types of evidence point to a role for *L. buccalis* in gingivitis, periodontitis and NUG. Just how important each of these genera are in the etiology of oral bacterial diseases remains unclear.

The role of *B. matruchotii* in periodontal disease may be as a contributor to calculus formation. Its relative importance in calculus formation *in vivo* is yet to be defined.

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Hepatitis, an Occupational Hazard

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Acute viral hepatitis is today a major world wide public health problem. In recent years approximately 70,000 cases were reported annually in the United States alone, which represents an incidence of about 33 per 100,000 population.⁵¹ Furthermore, this number only represents the top of the iceberg, since many persons who contract hepatitis have mild cases and do not seek treatment, and many cases are not reported to the proper agencies by physicians who treat hepatitis victims.

Several studies have shown that some occupational groups, particularly medical and health care personnel, risk greater exposure to viral hepatitis. These groups include physicians, surgeons, dentists, nurses, hygienists, laboratory technicians, and the staffs of: blood banks and institutions for the mentally retarded.³⁵ These health care personnel have a higher incidence of hepatitis than those who have no occupational exposure to patients or blood products.¹²

The incidence of hepatitis among dentists is of particular interest. One survey showed that 6.7% of dentists had a past history of hepatitis as

compared to only 2.4% of lawyers, the control group.¹⁸ In two other surveys an incidence of 7.2% and 4.5% of dentists asked, reported having had hepatitis.³⁷ These data indicate that dentists do acquire hepatitis more frequently than the general population.

Those dental specialists that are exposed to more blood, notably oral surgeons and to a lesser extent periodontists, have a much higher incidence of hepatitis, 21% compared to the general dentists with a 5% incidence.¹⁵ Published reports by Mosley and co-workers,¹² indicate that oral surgeons are at greater risk of contracting viral hepatitis than is any other professional group. Another alarming statistic is that the chance of a dentist contracting viral hepatitis increases the longer the dentist practices. To be more specific, a dentist who has practiced 30 years has a 20% chance of contracting hepatitis compared to 5% for all dentists. So the risk increases with the length of exposure.

When one speaks of viral hepatitis one is actually talking about two similar but epidemiologically distinct diseases.^{5,26} The initial designation of these two types, infectious hepa-

titis and serum hepatitis, was based on differences in their incubation period and apparent dependence of transmission of serum hepatitis on parenteral routes. In fact, it wasn't until the late 1920's that viral hepatitis was characterized as consisting of two types.⁵¹ The term viral hepatitis, type A, is synonymous with "infectious hepatitis," an ancient disease described by Hippocrates and long known as "epidemic jaundice," and "epidemic hepatitis." The term viral hepatitis, type B, is synonymous with "serum hepatitis," a disease with a more recent history. The first known outbreak occurred in 1883 among a group of shipyard workers who were vaccinated against smallpox with glycerinated lymph of human origin.⁹ Later, in venereal disease clinics and diabetes clinics, the disease was noted among patients infected from inadequately sterilized syringes and needles. Small outbreaks were also associated with serum used for measles prophylaxis and yellow fever vaccine.⁴⁷ In all of these instances, the vaccine had included human serum in its preparation.

In the early 1900's, investigators who engaged in epidemiologic studies of community outbreaks of hepatitis proposed a viral etiology for both types, A and B.³³ However, it wasn't until the 1940's that the viral etiologies were firmly established. This was achieved as a result of human volunteer experiments. Evidence of a filterable agent in blood and stools of patients with the disease was obtained.⁴⁹ Human experiments were required since it was impossible to propagate the causative agent of human hepatitis in laboratory animals.⁹ The experiments provided indirect evidence for the existence of at least two viruses.²⁷

In 1947, MacCollum suggested the names "virus A" and "virus B", the first being responsible for infectious (type A) hepatitis, and the second serum (type B) hepatitis.⁴⁵

Studies of the two types of hepatitis were hampered by lack of specific virologic methods to diagnose the two diseases and inability to transmit the diseases to laboratory animals. The symptoms and laboratory findings were so similar that they could not allow for differential diagnosis, although in epidemic outbreaks epidemiologic studies appeared to be helpful in differentiation. Today, viral hepatitis is separated into the two types on the basis of incubation period, epidemiology and more importantly on immunologic studies associated with the respective viral antigens.

Historically, viral hepatitis type A (HAV) has been considered to be an enteric infection similar to poliomyelitis, spread by close contact or by contaminated food or drink through a fecal-oral route.¹⁴ In addition to the classic fecal-oral route, HAV also may spread by inoculation of infectious blood.³⁴ After a dose related incubation period of from 15-50 days, there is an acute onset with fever, malaise, anorexia, nausea, and abdominal discomfort, followed within a few days by jaundice. Serum glutamic oxalacetic transaminase (SGOT) levels, a characteristic intracellular hepatic enzyme, becomes elevated, usually rising to a peak within 5-7 days. This rising phase of SGOT activity occurs during the preicteric (prejaundice) phase of the disease. Jaundice, when present, is detected at the time of peak transaminase levels. The period of elevated SGOT activity is usually transient, lasting 2 weeks, rarely persisting for more than 3 weeks. The disease

varies from a mild illness lasting 1-2 weeks, to a severe disabling disease lasting several months. Convalescence usually is prolonged. In general, severity increases with age, but complete recovery without sequelae or recurrences is the rule. The mortality rate is less than 1 percent. HAV is believed to be the cause of most large outbreaks of viral hepatitis. It is worldwide in occurrence; outbreaks are sporadic and epidemic, with a tendency to cyclic recurrences. Epidemics often evolve slowly, involve wide geographic areas and last many months. Outbreaks are common in families, institutions, summer camps, and especially among military troops. The clinical disease is most often manifest in children and young adults. The ratio of anicteric to icteric cases in adults is 1:1, and in children it may be as high as 12:1.³⁵ Studies of transmission indicate maximum infectivity during the latter half of the incubation period, continuing for a few days after the onset of jaundice or in anicteric cases, peak transaminase levels. During this period, the virus has been identified in serum, feces, urine and saliva.³

In 1973, Feinstein and associates, by use of immunoelectronmicroscopy techniques, demonstrated virus-like particles in the stools of volunteers infected with hepatitis A by both oral and parenteral routes.¹⁷ The isolated particles measure 27 nm in diameter, are serologically specific for this disease, and, as revealed by radioimmunoassay, antibodies to these particles have no immunologic relationship to hepatitis B antigens. The particles are aggregated by sera of convalescing patients with hepatitis A; furthermore, every patient with hepatitis A showed a positive serologic response to this antigen.¹⁵

Recently, sera or blood from infected individuals with hepatitis A have been shown to transmit the disease to marmoset monkeys, which develop a disease clinically identical to hepatitis A. Electronmicroscopy of thin sections of infected marmoset liver reveals intracytoplasmic virus particles, 27 nm in diameter, present usually in vacuoles of the cytoplasm. The hepatitis A virus resembles enteroviruses, a member of the picornaviruses.⁴⁰ These virus particles have been found in human liver cells as well.²⁰

Soon after the onset of the acute disease, patients with hepatitis A, but not those with type B, develop complement fixing and immune-adherence antibodies which persist for at least seven years after infection.³⁹ These studies also show that many people of low socioeconomic status have antibodies to the hepatitis A antigen, while persons of the upper socioeconomic levels have a much lower frequency of these antibodies. Furthermore, the frequency of occurrence of these antibodies increases with age.

Immunoelectronmicroscopic studies^{17,54} have shown that the hepatitis A antigen is present in the circulating blood for a short period of time prior to the onset of the disease. It is during this time that the disease may be transmitted by inoculation of the blood from an infected individual. With the onset of clinical disease and rise of antibody titer, the virus is quickly cleared from the blood and the individual is no longer infective. The hepatitis A antibody protects from reinfection with the same virus. However, the hepatitis A antibody does not protect the individual from infection with hepatitis B virus, or vice versa. In

other words, a single infection with hepatitis virus of either type A or type B confers homologous but not heterologous protection against reinfection.

Current understanding of the nature of the agents of viral hepatitis, type A and type B, strongly suggests that they are agents of altogether different groups. It appears that HAV is an unusual but essentially conventional virus which ultimately will be recognized as an enterovirus. The hepatitis B virus, due to its refractoriness to propagation in tissue culture, the production of high levels of particles in the blood stream, the body's inability to clear the virus from the circulation in many instances, and the long incubation period of the infection all may well be indicative of a group of viruses unlike the conventional animal viruses that we now know.³⁴

Viral hepatitis B has historically been regarded as being infectious only after parenteral inoculation of virus infected blood or blood products. However, it is now known that viral hepatitis B can be transmitted by means of the oral route,^{6,48,50} and by intimate contact involving non-parenteral or inapparent parenteral routes. It has been shown that as little as 10^{-4} ml of infectious plasma can produce clinical disease when injected into a recipient.^{6,9}

The distribution of type B hepatitis is worldwide. It occurs as an endemic infection with no seasonal trend and no particular predilection for any age group. There are definite high risk groups (as already pointed out) such as teen-agers, young adults, users of parenteral drugs (particularly addicts), individuals who have recently received blood transfusions, health care personnel, and

newborn infants born to mothers with type B hepatitis.

The typical course of viral hepatitis B begins with an incubation period of usually 45–160 days. The length of the incubation period appears to be dependent on the route of exposure—the parenteral route being shorter than the oral—and the size of the inoculum—the larger the inoculum the shorter the incubation period. Following an incubation period of usually 60 days, the SGOT values rise gradually, reaching peak levels approximately 30 days later. Jaundice when present occurs at this time. The patient may complain of painful joints for as long as 6 weeks prior to the onset of jaundice. Fever, if present, and anorexia are observed shortly before onset of jaundice. Abdominal pain and tenderness over the liver are also noted at this time. The SGOT levels may be elevated for several weeks to months before returning to normal. The ratio of anicteric to icteric infections has been estimated to be as high as 100:1.³⁵ Therefore, jaundice is a rare sign of hepatitis B.

As in the case of hepatitis A, most of what was known about hepatitis B, up to very recent times, was based on epidemiologic studies of human volunteers. A major breakthrough in our understanding of viral hepatitis B came in 1965, when Blumberg observed a foreign substance, initially called Australia Antigen, in the blood of an Australian aborigine. The investigation began as a consequence of the interest in inherited serum protein polymorphisms of blood. In 1961, Allison and Blumberg^{8,9} were examining the sera of patients who had received a large number of transfusions; they hypothesized that individuals receiving

multiple transfusions would receive some serum constituents different from those in their own blood and would respond by producing antibodies to these constituents. They were aware that both inherited and acquired antigens might occur in human serum and illicit antibody formation in blood recipients. Using a technique to detect both antibodies and antigens, Blumberg et al were able to describe a lipoprotein antibody to a complex system of inherited antigens analogous to the red blood cell groups. Shortly after the discovery of this lipoprotein antibody, they found precipitating antibodies against another antigen present in the same sera. These antibodies occurred in high frequency in patients with hemophilia and in others who received large numbers of transfusions. The antigen was first found in the serum of an Australian aborigine, and therefore, referred to as Australia antigen, Au(1).

The investigators discovered Au(1) was very stable when stored frozen. They also found that it was rare in Americans (0.1%), but quite common in people living in tropical and Asian countries. It was found that Au(1) occurred with greater frequency in leukemic patients. They decided to test the hypothesis that patients with Au(1) were more susceptible to leukemia. A corollary to this hypothesis is that patients with a high risk of contracting leukemia should have a high frequency of having Au(1) present in their sera. Patients who have Down's Syndrome are known to have a 20-100 fold greater risk of contracting leukemia than the general population. These investigators in fact found that patients with Down's Syndrome living in large institutions had a higher incidence of having

Au(1) in their sera than other mentally retarded controls in the same institutions or the general population.

The initial observation showed that Au(1) was persistent in the Down's Syndrome patients, adding support to their hypothesis. Those who were positive were persistently positive, and Au(1) did not develop in those who were persistently negative. In 1966, one of the Down's Syndrome patients who was initially negative for Au(1) was found to have a low Au(1) titer. While admitted for observation, the patient developed anicteric hepatitis. This finding was so striking that Blumberg et al immediately set out to determine if Au(1) is associated with viral hepatitis. The association of Au(1) with viral hepatitis was reported by Blumberg in 1967. This has since been confirmed by investigators in Europe, Japan, and the United States. The specific relationship of Australia antigen to viral hepatitis type B is now well established. The discovery and characterization of this antigen was a major breakthrough in hepatitis research. Even though the virus itself had not yet been identified conclusively, it permitted intensive study of the disease and the nature of the viral agent.³⁴

The Australia antigen, or hepatitis associated antigen (HAA), appears in serum during the course of infection with hepatitis type B virus. The antigenic reactivity is associated with 22 nm diameter spherical and tubular particles; the tubular particles are 200 nm long. Although organic solvents do not inactivate viral infectivity, ether significantly reduces the diameter of the HAA spherical particles, converts the tubular particles to small spheres, and increases the bouyant density of

HAA.³⁴ These data imply HAA contains lipid (soluble in ether), and suggests that the antigen is not identical with the infectious viral particles. Purified HAA particles contain two major and one minor protein and an outer 2 nm layer of lipid which is digested by ether, but neither DNA nor RNA.³⁴ The absence of nucleic acid in the HAA particle supports the suggestion that this particle represents the protein coat of the infectious viral particle. Therefore, the HAA antigen has been renamed hepatitis B surface antigen (HBsAg), with the antibody to this antigen anti-HBs, or HBsAb.

Larger 42 nm double shelled spherical particles, often referred to as Dane particles (after their discoverer), are observed less frequently. These particles are more complex. The 7 nm outer surface or envelope contains HBsAg and surrounds a 27 nm inner core which does not react with anti-HBs. This core particle, however, contains its own antigen, called the core antigen (HBcAg). Convalescent hepatitis serum, free of anti-HBs, has been demonstrated by immunoelectronmicroscopy to aggregate the core particles released by treating the Dane particles with detergent.²¹ This antibody, distinct from anti-HBs, is now known as anti-core antibody, or anti-HBc. Further electronmicroscopic studies have shown the presence of particles resembling the cores in the nuclei of infected liver cells. This has been verified by the use of fluorescein conjugated anti-HBc, which produces nuclear fluorescence when the core particles are present. In addition, fluorescein conjugated HBsAb produces fluorescence in the cytoplasm of these infected cells.⁴

Staining properties of the inner core of the Dane particle indicates the presence of nucleoprotein, and DNA polymerase activity.²⁵ Recently, circular double-stranded DNA with a molecular weight of 1.6×10^6 daltons (smaller than any double stranded DNA of any known virus) has been isolated from highly purified Dane particles.⁴⁵ These observations strongly suggest that the Dane particle is the complete virus of hepatitis B, with the core being the nucleocapsid, and the surface or outer protein coat, the hepatitis B surface antigen. One concludes from these studies that the core component is produced in the liver cell nucleus and the coat material in the cytoplasm of infected cells. The core component is enveloped by the coat material and released by the cell as the complete Dane particle or hepatitis B virus. In addition, the infected hepatocytes produce excess viral coat lipoprotein material. It is excreted by the hepatocyte and circulates in the blood stream as the 22 nm particles, the HBsAg.¹³ The amino acid composition of the HBsAg has been determined, and it differs significantly from that reported for mammalian cells and from that of other DNA and/or RNA viruses. Disulfide bonds play an active role in maintaining the antigenic integrity of the HBsAg. The synthesis of these virus specific proteins are specified by the DNA of the HB virus core particle. In addition, the Dane particles have the ability to synthesize DNA from an endogenous template, as shown by purified core components which contain DNA polymerase.²⁵

Recently, another antigen, designated the e-antigen, has been described which is distinct from

HBSAg and HBcAg. It has been reported in some HBsAg—positive sera, but not in negative sera, and appears to be specific for hepatitis B infection.³¹ Circulating e-antigen is structurally distinct from HBs and HBc particles.³² Epidemiologic evidence suggests that e-antigen may be a viral gene product whose presence in serum is associated with infectivity and liver pathology.³² In patients with acute hepatitis, the presence of e-antigen has been reported as a possible marker of later development of chronic hepatitis and continued infectivity of the patient's serum.³³ Preliminary results have shown a large number of Dane particles in serum samples containing the e-antigen; whereas high titers of antibody to the e-antigen are found in healthy individuals in whom the Dane particles cannot be demonstrated, but in whom the HBsAg can be demonstrated. This strong correlation between the presence of the e-antigen, Dane particle, and high level of DNA polymerase activity strengthens the hypothesis that the e-antigen is a marker of hepatitis B virus activity or the infectivity of a given serum.

In summary, the complete hepatitis B virus (HBV) is believed to be the 42 nm Dane particle, composed of a 27 nm core (HBcAg) and a 7–8 nm antigenically distinct coat or hepatitis B surface antigen (HBsAg). The HBsAg reactivity also characterizes the much more numerous 22 nm diameter spherical and tubular particles which represent excess virus coat material excreted by the infected hepatocyte. Inside the Dane particle core is a DNA-dependent DNA polymerase and a double-stranded, circular DNA molecule, the presumed viral genome. An

e-antigen is also associated with the virus, but its location and nature has not been identified. It appears to be intimately associated with the pathogenesis of liver damage, as well as infectivity. The virus replicates only in liver cells; the cores are produced in the nuclei and the outer coats in the cytoplasm. The coat material is produced in excess of cores and always forms the bulk of the particles present in the serum, however, the infectivity of the serum is dependent on the number of Dane particles present.

In patients with hepatitis B, apart from the changes in the serum levels of bile pigments and liver enzymes (transaminases) consequent to liver damage, the various virus particles appear, the viral enzyme DNA polymerase becomes detectable, and the production of antibodies to the viral antigens is stimulated. Circulating HBsAg can usually be detected in the serum of patients several weeks before the onset of clinical hepatitis B, and persists until convalescence in the uncomplicated disease, disappearing by the sixth month after exposure. It is of interest to note that the disappearance of circulating HBsAg occurs following the regular appearance of a cell-mediated immune response to HBsAg.²⁴

Some weeks or months after recovery, anti-HBs generally, but not invariably, develops, usually at low levels detectable only by sensitive assay methods. It facilitates the removal of the virus particles from the circulation.⁴⁷ During this phase circulating antigen-antibody complexes can be detected by electronmicroscopy. Following removal of the HBsAg—HBsAb complexes, HBsAb titers rise to high levels and persist 3–5 years. There is a growing body of

evidence to suggest that high titers of HBsAb afford protection from infection by the hepatitis B virus.⁵³

Antibody to HBcAg develops several weeks after the onset of the appearance of the antigen and well before the appearance of HBsAb. It persists following recovery with a slow decline in titer. It is closely associated with the onset of clinical symptoms.⁵⁴ Because it appears earlier than anti-HBs and is present for a shorter period of time, it is a possible marker of ongoing or recent HBV infection in the HBsAg-negative patient.²¹ The anti-HBc antibody does not signal recovery, and does not seem to influence the course of the disease. It is merely produced in response, and it indicative of, active viral replication in the liver.²²

The DNA polymerase, associated with the core, becomes detectable in the serum when sufficient Dane particles are circulating. It attains peak levels at the time of peak viremia.³⁸ Being transient in acute disease, it appears early in the period of surface antigen reactivity and disappears when or before HBsAg does. Since its activity seems to correspond to the presence of large numbers of Dane particles in the serum, quantitation of DNA polymerase may prove useful in distinguishing between highly infectious sera and sera of low infectivity containing HBsAg.

The immune response following infection with hepatitis B virus elicited by the three distinct antigenic components of the infectious agent: HBsAg, HBcAg, and e-antigen in quite variable. These include (1) late seroconversion to anti-HBs, without detectable liver damage or without a stage of circulating HBsAg

or anti-HBc, (2) the development of transient anti-HBc without detectable HBsAg, followed by anti-HBs, (3) variable appearance and reappearance of HBsAg, with or without clinical disease (the carrier state).²³ The first, or subclinical infection, is probably the most common type, since most persons with circulating anti-HBs do not possess a known history of hepatitis.

The view that the hepatitis B virus exerts its damaging effect on hepatocytes by direct cytopathic changes appears inconsistent with the persistence of large numbers of HBsAg and HBcAg in the liver cells of a significant proportion of healthy persons carrying the HBsAg in their blood. Consequently, investigators have proposed an immunologic explanation for the pathogenesis of the lesions found in association with hepatitis B. One possibility is that HBsAg-HBsAb complexes form on the surface of infected hepatocytes and activate the complement system, resulting in hepatocellular damage. Substances released from these damaged hepatocytes and various components of the complement system act as chemotactic factors for the influx of leukocytes. These leukocytes release their lysosomal enzymes resulting in further hepatocellular damage. This damage could be further aggravated and perpetuated by circulating HBsAg immune complexes.⁵³ The identification of these immune complexes by electronmicroscopy, and the common presence of joint pain and other serum sickness symptoms characteristic of immune complex disease several weeks prior to the onset of clinical hepatitis are supportive of this mechanism. However, the cell mediated immune response appears to play a more important

role. The acute stage of the disease is closely associated with the development of cell-mediated immunity to the hepatitis B antigens. Such immunity persists during convalescence, and disappears after recovery. Finally, such immunity is absent in the HBsAg carrier state. This data is consistent with the hypothesis that cell mediated immunity may be involved in terminating the viral infection and, under certain circumstances, promoting hepatocellular damage. It appears that immune lymphocytes (T-lymphocytes) recognize the viral antigens on the surface of infected hepatocytes and play the primary role in induction of acute hepatic disease. They are also more responsible for the severity and persistence of cell damage than are the humoral antibodies.⁷

Evidence of sensitization to a liver-specific lipoprotein has been shown to be important in the progressive hepatic damage of chronic active hepatitis following HBV infection.¹⁶ It appears that host proteins are a part of the HBsAg. Suitable treatment of purified HBsAg leads to the liberation of a number of serum proteins as well as viral proteins.³⁶ Animals hyperimmunized with purified preparations of HBsAg produce antibodies to human serum proteins as well as to the viral proteins. It, therefore, seems that human serum proteins are an integral part of the HBsAg.⁵² It is believed that the cell-mediated immune response to hepatitis B virus initiates an autoimmune reaction to liver specific lipoprotein on the hepatocyte surface, and this reaction is responsible for hepatic damage.²⁸ In the absence of the T-lymphocyte response, the autoimmune reaction does not occur, and the virus is able

to establish a symbiotic harmless relation with the host.²⁸ Although there is at present no convincing evidence that a humoral or cell-mediated immune mechanism is involved in all the clinical patterns of viral hepatitis, there is considerable evidence to suggest that the course of the hepatitis is greatly influenced by whether the immune response eliminates the virus from the liver or not. In typical viral hepatitis B, HBcAg are cleared from the liver along with the appearance of characteristic histologic lesions. Elimination of these is associated with a self-limited course of the disease. In the chronic carrier state, there is inadequate anti-HBs production resulting in elimination of the core particles from the nucleus, but persistent surface antigen in the cytoplasm. In chronic persistent hepatitis there is impaired antibody response to HBcAg. The HBcAg persists in the hepatocyte nucleus, and minimal evidence of hepatocellular injury is observed histologically. Chronic active hepatitis is characterized by inadequate anti-HBs and anti-HBc. Both antigens persist with continued hepatic injury probably produced by the cell-mediated immune response. Finally, fulminant hepatitis is believed to be due to excessive antibody response with the development of immune-complex disease and widespread hepatocellular destruction.¹¹

According to Reed et al,⁴² a balanced immune response is essential for complete recovery from viral hepatitis B. This involves the destruction of the infected hepatocytes, release of the intracellular virus, complexing of the free virus with antibody and clearance of the complexes by the reticuloendothelial system. This fortunately is the com-

non course of events. Rarely there is a defect, either qualitative or quantitative, which results in persistence of HB antigenemia and a varying degree of immunologically-mediated hepatic damage.

As a result of the development of sensitive tests for the detection of HBsAg and anti-HBs, successful transmission of hepatitis B to non-human primates has been accomplished in rhesus monkeys and in chimpanzees.^{15,41} Hepatitis B infection in chimpanzees resembles infection in man, and, like in man, is characterized by a spectrum of host responses ranging from inapparent to frank hepatitis. Chimpanzees appear to be highly susceptible to HBV and serve as a sensitive indicator of the presence of the virus; they serve as useful models for studies of the host response to the virus. In contrast, infection of rhesus monkeys is entirely inapparent. Rhesus monkeys are quite resistant to HBV infection. By establishing standardized pools of hepatitis B virus of known infectivity titer in these primates, it is hoped that researchers can characterize the host response, evaluate various aspects of passive immunization against hepatitis B infection, and eventually develop an inactivated hepatitis vaccine.⁴¹

Until the advent of a proven vaccine or other immunization or prophylactic therapy, the dentist (especially the oral surgeon) and other health professionals at high risk must protect themselves, first against contracting the disease, and second against passing the disease to others. Individuals who have had hepatitis A are only infectious for a brief period of time closely associated with the clinical disease. The chronic

carrier state of HAV has not been identified.

Unfortunately, the situation with hepatitis B is not the same. There are many persons who can transmit the infection long after they have recovered from the clinical disease, or who may never have had any symptoms of hepatitis at all. Patient histories cannot be relied upon to screen out all those who are potential sources of hepatitis, as only analysis of the blood can determine carriers. The carrier state exists when there is a persistent HBV antigenemia in the absence of symptoms, often but not always in association with an ongoing detectable liver pathology.⁴³ It is generally assumed that those who have HBsAg in their blood are potentially infective. The carrier state may occur in about 5–10% of acute infection with clinical symptoms, and in an unknown percentage of subclinical infections. In carriers, HBsAg is present in the blood for many years and probably will remain there indefinitely.⁵⁵ DNA polymerase is usually demonstrable, showing that Dane particles are also circulating. The continued replication of the virus in the liver, indicated by very high titers of HBcAg, is usually present in the carrier. The condition of the liver itself varies from being apparently normal to undergoing chronic aggressive hepatitis. Anti-HBs fails to appear in the carrier; moreover, administration of serum containing high titers of anti-HBs does not affect the carrier state.⁴⁷ However, high titer anti-HBs serum given to normal individuals may provide passive protection against hepatitis B infection and in infected individuals induce recovery.⁵³

There are several high risk groups of carriers within the population. These include 45% of the drug addicts.³⁰ Patients who have received multiple transfusions, renal dialysis patients, those with immunologic defects, patients on immunosuppressive drugs, Down's Syndrome victims (especially institutionalized), patients with leprosy, and lymphatic leukemia all have a higher incidence of HBsAg in their blood than the normal population. Also, while 1% of the population of the United States and Europe are HBsAg-positive, in parts of Asia and Africa the rates may be as high as 20%. Patients who have recently emigrated from Africa and Asia should be held suspect.

The proportion of dentists who have HBsAg in their blood is somewhat higher than that of the general population and about the same for hospital workers and physicians.² Of prime concern is whether these dentists with antigenemia pose a hazard to their patients. Circumstantial evidence that hepatitis can be acquired in the dental office has been available for many years.¹⁹ There have been several reports linking clusters of cases of HBV to initiation by dentists. In May, 1974, Levin and Wands²⁹ reported 13 cases of hepatitis B traced to a dentist who had recovered from HBV but who remained HBsAg-positive. Also, in 1974 there was a report of 53 cases of HBV traced to an oral surgeon who was an asymptomatic HBsAg-positive carrier.⁴⁴ In both these situations, no inadequacies in instrument sterilization were found. Obviously each of these practitioners retired from clinical practice. However, there are many reports of HBsAg-positive dentists who have

not transmitted the disease while treating patients. Consequently, there has been no suggestion that dentists and other health professionals who are HBsAg-positive should cease practice. The level of antigenemia and the intensity of contact required to transmit the disease are unknown factors. Some evidence indicates that not everyone whose serum is HBsAg-positive necessarily disseminates the disease.¹ A paper in the *New England Journal of Medicine*, 1975,² reported on a prospective study of numerous contacts with HBsAg-positive health workers. The study indicated that health workers who are acutely or chronically HBsAg-positive are not an undue risk to their patients and, therefore, they should not be restricted in performing their normal professional duties. Nevertheless, although carriers of hepatitis B do not present as great a risk to contacts as once believed, the possibility of transmitting the disease is still existent. Until methods are developed for distinguishing carriers who are likely to transmit hepatitis B from those who are not, we should not treat all carriers as infectious.¹⁰ However, if there is incontrovertible evidence that a health professional has transmitted the disease to his patients, he cannot be permitted in clinical practice.

There may be wide differences in infectivity between those incubating the disease and carriers, and between those who are symptomless carriers and those with various degrees of progressive hepatitis. Research now centers around estimation of DNA polymerase and the e-antigen. DNA polymerase is indicative of the number of Dane particles circulating, and the e-antigen has been corre-

lated with the degree of infectivity. As yet, techniques to monitor DNA polymerase and e-antigen are not now widely available. It is hoped that such assays will be capable of separating infectious HBsAg carriers from noninfectious carriers.

Prevention of transmission of hepatitis from an infectious individual is difficult, but not impossible. Sterilization of all instruments using viricidal methods or using disposable instruments eliminates transfer of the hepatitis virus by this route. This includes all instruments that may come in contact with blood or saliva. Several agents and methods are available for effective sterilization. These include immersion in boiling water (100°C) for 30 minutes, or exposure to saturated steam for 30 minutes at 121°C and 15 psi pressure, or dry heat 160°C for one hour, or ethylene oxide gas 10% in carbon dioxide at 55–69°C for eight to ten hours.¹² These procedures destroy the entire virion. However, based on the rationale that any procedure which destroys the surface coat (HBsAg) will eliminate viral viability, the Center for Disease Control in Phoenix, Arizona has recommended several methods. These include immersion in boiling water (100°C) for 10 minutes, or immersion in 1% sodium hypochlorite solution for 10 minutes. In addition, sporicidal disinfectants, it is believed, will destroy the hepatitis B virus. These include alkaline glutaraldehyde, (Cidex) and ethylene oxide. Solutions of isopropyl alcohol 70–90% and quaternary ammonium compounds are not considered to be effective.

The most effective means to prevent dentist-patient or patient-dentist transmission is by the use of disposable gloves. In most studies of

cross-infection in hospitals, hands have been shown to be a common route of transmission. There is no evidence that thorough washing will remove or inactivate the hepatitis B virus. There are no known germicidal soaps which will inactivate the virus. Furthermore, the repeated washing of the hands causes roughness, cracking, and chapping of the skin which provides a ready route of infection. The solution is obvious: avoid contamination of the hands by simply wearing disposable sterile gloves, especially when the hands are likely to be exposed to blood.

Feldman,¹⁸ in 1975 reported that few dentists take adequate precautions to lessen the risk of transmitting or acquiring hepatitis. Few wear gloves even for surgical procedures where hemorrhage is likely. Oral surgeons frequently get splattered with blood across their face and clothing, an obvious method of transmission of the disease. Few wear face masks. It is no wonder oral surgeons have the highest incidence of hepatitis of any health profession.

It is remarkable that only ten years ago Australia antigen was detected and its association with viral hepatitis documented. Our knowledge of viral hepatitis, especially type B, has grown enormously in this brief period. At present, there is active research to develop a vaccine or other prophylactic measure which will eliminate the health hazard of viral hepatitis. Until that time, we dentists have an obligation to ourselves and our patients to take precautions against viral hepatitis. Sterilization of all instruments, the use of disposable gloves and face masks, especially when working in bloody fields, are simple, inexpensive, and effective methods now available.

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**Prosthetic Obturation Subsequent to Total
Resection of the Soft Palate
A Comparison of Two Case Histories**

W. O. RAMSEY* AND E. P. QUARANTILLO**

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INTRODUCTION

Two patients, simultaneously referred by separate practitioners, presented remarkably similar surgical defects of the soft palate. This article presents for comparison those factors which influenced the choice of treatment procedure for each patient and summarizes the procedures utilized in constructing appropriate prostheses. A subjective comparison of two types of obturator is made.

CASE HISTORY No. 1

A sixty-three year old, white, female patient, completely edentulous, was referred for complete denture construction three years subsequent to total excision of the soft palate and bilateral removal of the tonsillar pillars. Previous attempts to provide complete dentures and a horizontal obturator had failed because of limitations in the degree of mandibular opening and a constriction of the orifice of the mouth. The patient had been wearing only a maxillary complete denture with an ineffectual horizontal extension of resin into

the pharyngeal region (Fig. 1). Speech was unintelligible. Food and fluids were discharged through the nostrils during swallowing.

Clinical examination revealed resection of the soft palate at approximately the vibrating line between movable mucosa of the soft palate and attached mucosa of the hard palate. A zone of tissue suitable for a posterior palatal seal remained. Attachments of right and left tensor and levator palati muscles and right palatoglossus muscle to the palate had been severed. The hamular process of the right medial pterygoid lamina was absent. An interridge denture space of 15 mm., measured in the midline, was available. Basal seat tissues for complete dentures were of normal size and quality. Saliva was scanty and somewhat serous and the patient complained of persistent dryness of the naso- and oropharynx. History of postsurgical radiation was given.

Complete dentures were constructed by conventional means and

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FIGURE 1. Ineffective appliance originally worn by patient in Case History I.

a period of three weeks was permitted for denture adjustment and patient adaptation prior to construction of an obturator.

Two approaches to obturation were considered: a vertical, meatus-

type appliance and a fixed horizontal appliance (Fig. 2). Although the palatal defect afforded excellent access for a vertical obturator, this type of appliance was not elected in this particular patient for the following reasons: the vomer, in-

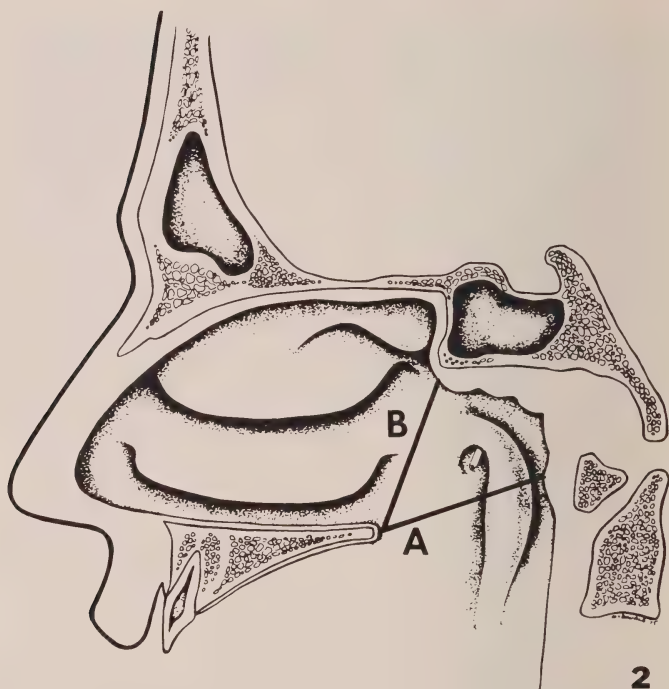


FIGURE 2. Diagrammatic representation of two types of obturator, indicating directional placement of appliance in relation to hard palate and pharynx. A = Horizontal appliance; B = Vertical appliance.

ferior and middle conchae (turbinates) extended posterior to the line of resection; the tori and orifices of the internal auditory (Eustachian) tubes would be covered; and, configuration of a vertical appliance would render insertion and removal difficult in view of the limited oral opening available.

Based upon the following observations, a fixed horizontal appliance

appeared practical. Examination of the nasopharynx and oropharynx revealed a distinct elevation of the superior fibers of the superior pharyngeal constrictors, possibly combined with unresected horizontal fibers of the palatopharyngeus muscle.¹ These muscle fibers formed a continuous band, circumscribing and bulging into the lumen of the pharynx. (Figs. 3, 4). This encircling band of

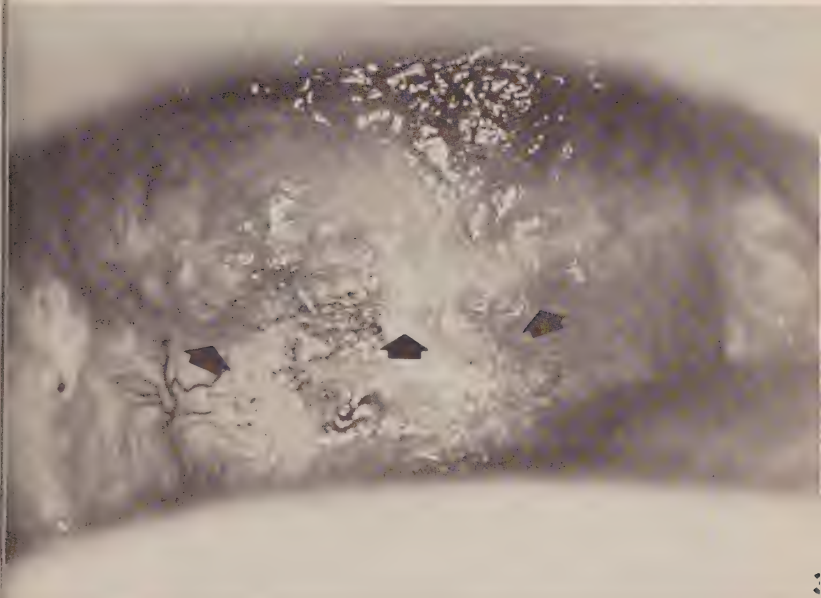


FIGURE. 3. View of pharynx (Case History I). Arrows indicate the circumferential band of contractile tissue in resting form against which horizontal obturators were constructed in Case Histories I and II.

tissue passed just inferior to the orifices of the internal auditory tubes and terminated anteriorly on the right and left sides in the region of the medial pterygoid laminae. The levator muscle bulge was discernible below each internal auditory tube and the salpingopharyngeus muscles exhibited a slight contractile bulge. During attempts at phonation, a significant medial bulging of these muscular structures occurred,

constricting the lumen of the pharynx. This constriction was similar in form and location to a classical Passavant's Ridge (Figs. 5, 6), differing only in the fact that it completely circumscribed the pharynx and did not appear subject to the rapid fatigue characteristic of Passavant's Ridge.

A 15 gauge stainless steel loop was attached into the palatal surface²



FIGURE 4. Circumferential band of Fig. 3 in fully contracted state.

of the maxillary complete denture (Fig. 7); the loop was adjusted to approach the pharyngeal band of contractile tissue; a wafer of impression compound was molded over and within the loop and border molded with a mouth temperature softening impression wax (Figs. 8, 9, 10, 11). Functional speech, swallowing of liquids and forced retraction, depression and lateral rotation of the head were employed to produce border

molding, permitting an effective seal during function and providing a patent airway during relaxation. A marked improvement in speech was immediately apparent and deglutition was possible without nasal discharge. The molded obturator was reproduced in acrylic resin (Figs. 12, 13). Subsequently, a hollow bulb was added to the superior surface of the obturator in order to further modify voice quality.

CASE HISTORY No. II

Concurrent with treatment of the previously described patient, a fifty-two year old, white, female patient was referred for construction of complete dentures and an obturator

four months subsequent to excision of the entire soft palate. Ill-fitting dentures constructed prior to the patient's surgical experience were still being worn. No problems existed in regard to jaw mobility, jaw relations, basal seat tissues, denture



FIGURE 5. A cleft palate patient exhibiting a classical example of Passavant's Ridge on posterior wall of pharynx. The ridge is depicted in its relaxed state posterior to the posterior-inferior border of a fixed horizontal obturator.

space or saliva. Speech was barely intelligible and hypernasal. Food and fluids were discharged thru the nostrils during swallowing.

The posterior tonsillar pillars had been excised; however, the palatoglossal arches remained intact. Approximately 10 mm. of fibrous,

scarred soft palate remained distal to a zone suitable for a palatal seal. The vomer and conchae lay anterior to the line of palatal resection.

Clinical examination of the oral and nasal pharynx revealed a horizontal band of muscular tissue almost identical to that presented by the



FIGURE 6. Passavant's Ridge of Fig. 5 in fully contracted state sealing against the posterior-inferior border of fixed horizontal obturator.



FIGURE 7. Preparation of a maxillary complete denture to receive a stainless steel loop which will serve as a scaffold for the impression material and completed obturator. The loop is attached with autopolymerizing resin into the denture base, after which it may be cut and or adjusted by bending to the desired height and degree of pharyngeal clearance.



FIGURE 8. Superior view of impression for horizontal obturator.



FIGURE 9. Lateral view of impression for horizontal obturator.

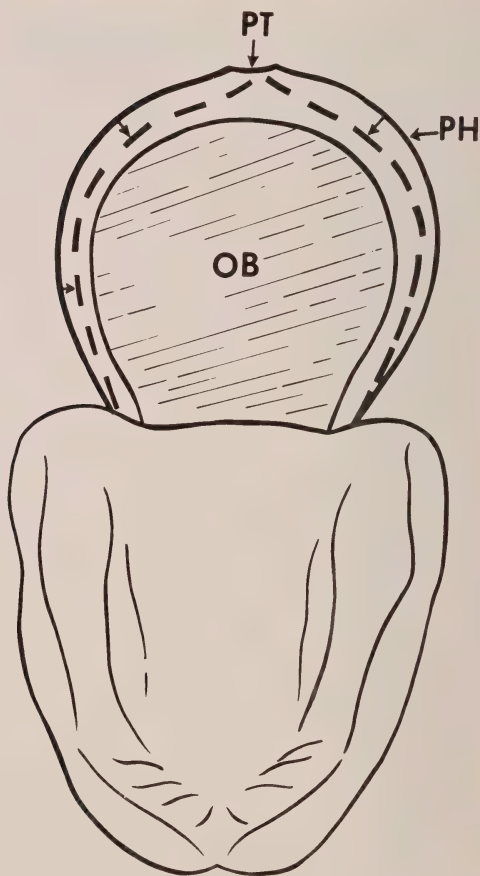
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FIGURE 10. Superior diagrammatic view of horizontal obturator in relation to pharynx. OB = Obturator; PH = Pharyngeal wall in resting position (Solid Line) and in contraction (Dotted Line) as it seals against obturator; PT = Pharyngeal Tubercle of occipital bone.



FIGURE 12. Superior view of completed horizontal obturator. Case History I.



FIGURE 13. Lateral view of completed horizontal obturator. Case History I.

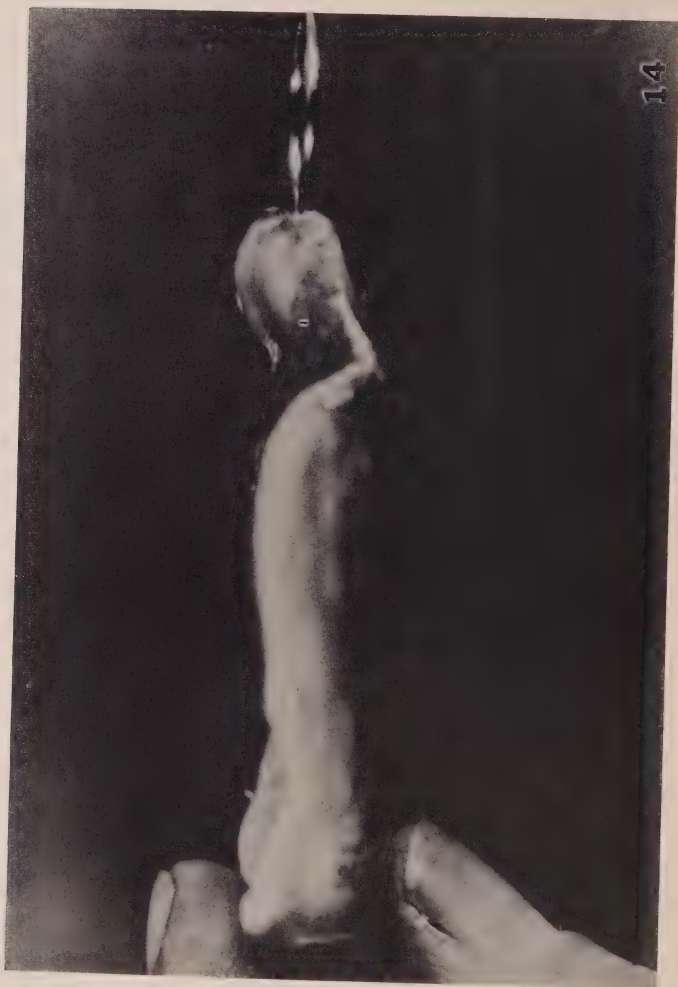


FIGURE 14. Loop attached to trial base in preparation for impression on trial base for horizontal obturator of Case History II.



FIGURE 15. Loop attached to trial base in preparation for impression on trial base for vertical obturator of Case History II. Lateral view.



FIGURE 16. Completed vertical obturator, superior view. Case History II. Note perforation of obturator providing nasal cavity drainage, airway and speech quality.



FIGURE 17. Completed vertical obturator, lateral view. Case History II.

Subsequent to evaluation of the diagnostic appliances, a vertical obturator was constructed upon the recently inserted maxillary complete denture (Figs. 16, 17).

HORIZONTAL VS. VERTICAL APPLIANCE

From the viewpoint of the prosthetist, the following comments are offered in regard to the appliances constructed in Case History II:

In Case History II, both appliances were effective in restoring normal deglutition and speech. However, the vertical obturator provided better clarity of speech with a minimal amount of operating time and effort. (It was necessary to add a hollow bulb to the superior surface of the horizontal appliance in order to fully restore voice quality.) Because of the low degree of contractile activity in the circumferential bulge of pharyngeal musculature, the horizontal appliance required considerable precision in placement and molding; also, the space between the periphery of the appliance and the resting form of the pharynx was so limited as to produce difficulty in breathing thru the nose, especially after the collection of mucus upon the appliance. Neither appliance appeared to affect retention of the trial base.

From the viewpoint of the patient, the vertical obturator was the appliance of choice. In comparing the two appliances, the patient experienced less difficulty in adapting to the vertical appliance, greater ease in swallowing, breathing, and speaking, and a lesser awareness of the presence of the obturator. She also commented upon an improvement in auditory acuity accompanying the

disappearance of a "stiffness" of the ears after wearing the appliance.

In comparing the effects upon speech of the horizontal obturator of Case History I, with the two trial obturators of Case History II, the following observations are offered:

The quality of speech sounds after obturation of Patient I (horizontal appliance) was approximately equal to that provided by a horizontal appliance in Patient II. However, the vertical appliance in Patient II provided a decidedly superior voice quality and a markedly greater degree of patient acceptance of the appliance.

SUMMARY

Two case histories have been presented summarizing treatment of two patients presenting almost identical total resections of the soft palate. Factors influencing obturator design and zones of pharyngeal seal or occlusion were discussed. A subjective comparison was made of two appliances (horizontal and vertical) constructed for one of the patients.

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The authors gratefully acknowledge the assistance of Mr. Richard Elliott, Mr. Jerry Gadd and Mr. Stephen Lipscomb, Department of Educational and Instructional Resource of the Dental School, in the preparation of illustrations for this manuscript.

I. Determination of the Ideal Time of Enamel Conditioning for Sealing Proximal Surfaces of Teeth

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INTRODUCTION

The use of phosphoric acid for conditioning enamel surfaces to insure bonding of plastic materials to tooth enamel was first suggested by Buonocore (1955). The conditioning procedure is now considered a basic step and is commonly referred to as the "etching technique" (Buonocore, 1971, 1972).

The length of time that the acid was allowed to remain in contact with the enamel surface was considered to be critical. When using a liquid acid conditioner (50% phosphoric acid plus 7% zinc oxide by weight), many investigators recommended an etching period of 60 seconds duration. It was additionally suggested that the conditioning solution be applied with a small cotton pellet using a gentle movement to and fro (Buonocore, 1955, 1970, 1971; Gwinnett, 1971; Davila, 1972; Davila, Buonocore, Greeley and Provenza, 1974).

Application of an enamel conditioner and a self-polymerizing plastic material to the proximal enamel surface has been studied recently. Both the conditioner and plastic were placed in contact with the enamel surface by means of a special plastic carrier (Davila, Sisca, Tinanoff and Provenza, 1975). The conditioner in the carriers was in the form of a gel.

Because of differences in the chemical and physical characteristics of the enamel conditioner as well as differences in its application technique, it was suspected that the interval of time for conditioning should be investigated.

The purpose of this *in vitro* study is to determine the most desirable conditioning period when the gel is applied to proximal surfaces of teeth via a plastic carrier.

MATERIALS AND METHODS

Ten non-carious molar teeth for each experimental group were se-

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lected and stored in 10% neutral buffered formalin. The teeth were subsequently cleaned with pumice using a rotary bristle brush and stored in distilled water under refrigeration.

The specimens were placed in a tooth holder and secured in position to simulate the *in vivo* relationship between adjacent teeth (Davila et al., 1975). Application of the conditioner and plastic resin (50% phosphoric acid in a gel base* without zinc oxide; self-polymerizing plastic material**) to proximal surfaces of teeth was accomplished with a plastic carrier.

Three different etching periods (60, 90 and 120 seconds) were studied. Evaluation of optimal etching time by each of two observers was determined by studying the number, uniformity and shape of the tags formed in the process of plastic infiltration of the enamel pores. Specimens were examined at varying magnifications on a AMR-1000 Scanning Electron Microscope at 20 KV.

RESULTS

The inner surface of the plastic coatings, recovered from completely demineralized enamel surfaces etched for 90 and 120 seconds, exhibited tags of varying formation. All the 60 second etched specimens, on the other hand, did not reveal appreciable tag formation when infiltrated with the plastic resin.

Scanning electron micrographs of the ten specimens etched for 90 seconds and infiltrated with self-polymerizing plastic material illustrated a uniform distribution of numerous tags (Fig. 1). The overall pattern of the replica reflected a honeycomb appearance resembling the prismatic structure of enamel (Fig. 1). Tags in specimens etched for 90 seconds were longer than those etched for 120 seconds and were more robust (Fig. 1, inset).

The inner aspect of the ten plastic coats, representing the replicated 120 second etched enamel surfaces, exhibited more shallow pores and fewer honeycombed areas (Fig. 2). Accordingly, the tags in these specimens were of irregular shapes and varying lengths (Fig. 2, inset).

DISCUSSION

Because the proximal surfaces of teeth in the oral cavity are less accessible and because the etching procedures for exposed enamel surfaces are not applicable for adjacent surfaces (Buonocore, 1955, 1970, 1971; Gwinnett, 1971), modification of the etching technique was necessary: a gel base conditioner was substituted for a liquid; a plastic carrier was substituted for a brush or cotton pellet; and duration of etching was varied.

Using a 50% solution of phosphoric acid plus 7% by weight of zinc oxide, Buonocore, (1955, 1970, 1971) and Gwinnett, (1971) recom-

* Direction Sealants, Tp Laboratories, Inc., LaPorte, Indiana 46350.

** Concise Enamel Bond System, 3M Company Dental Products, 3M Center, St. Paul, Minnesota 55101.

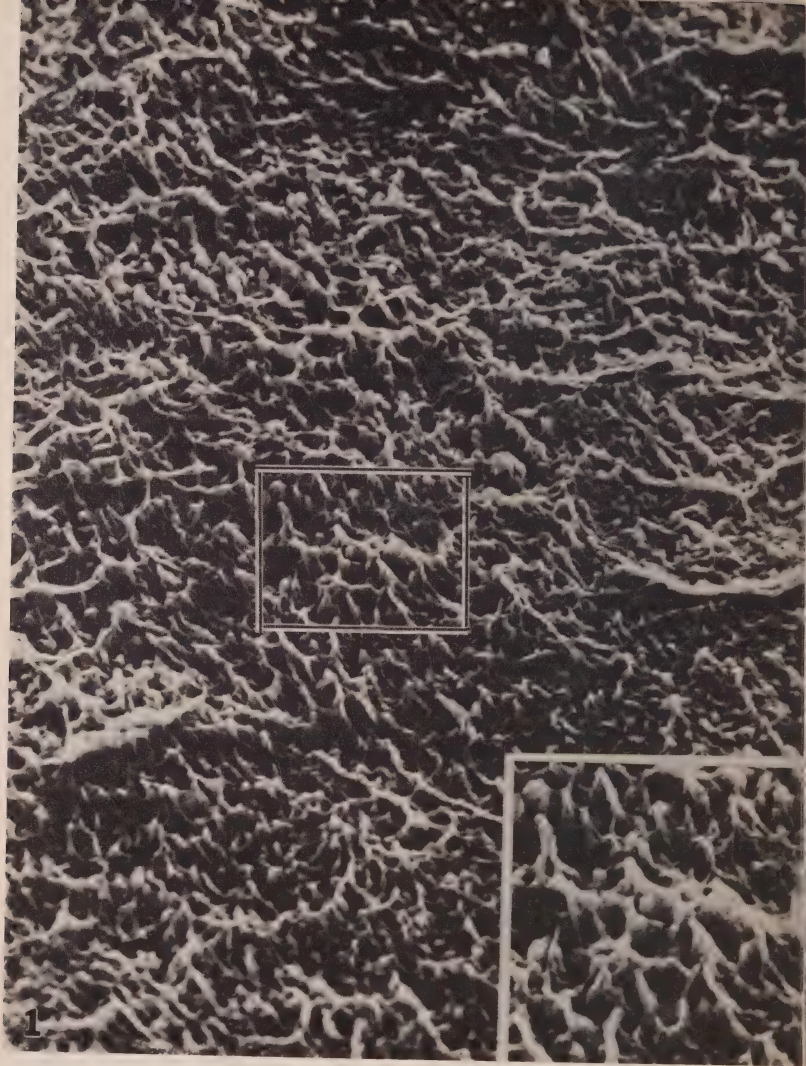


FIGURE 1. Scanning electron micrograph of a specimen infiltrated with a self-polymerizing plastic material. The tags (black arrows) appear generally dispersed over the surface. Note honeycomb areas (white arrows). Enamel conditioning time: 90 seconds.

X400

FIGURE 1, inset. Increased magnification of tags which were long and robust.

X660

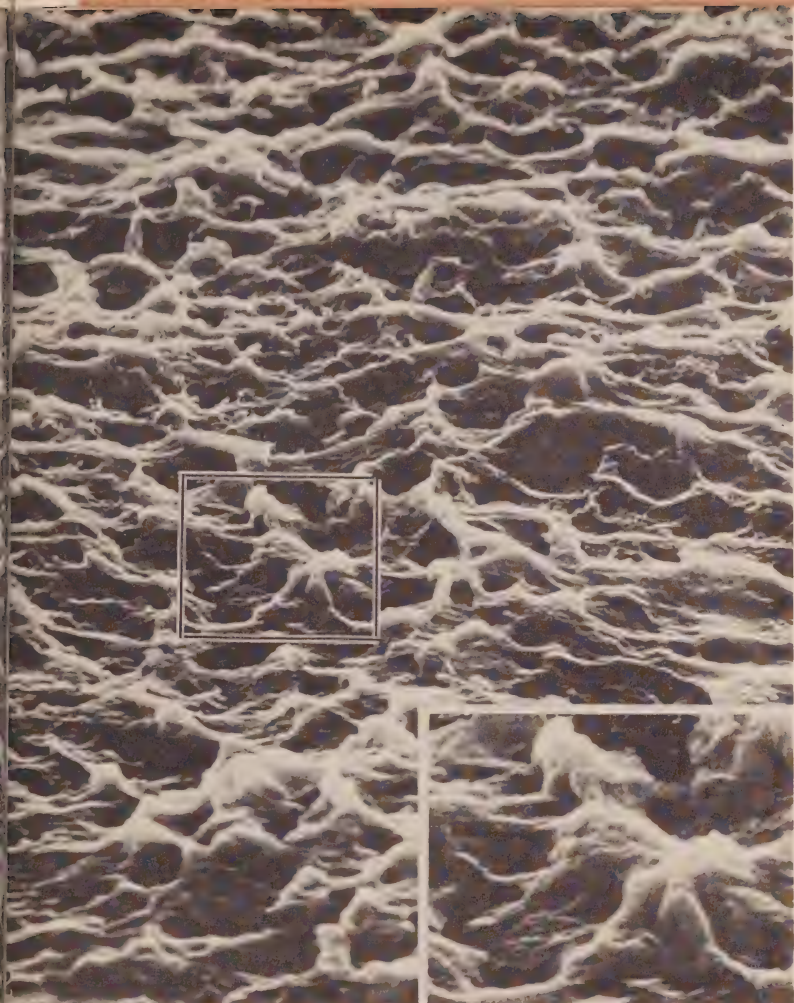


FIGURE 2. Scanning electron micrograph of a specimen acid etched for 120 seconds and infiltrated with a self-polymerizing plastic material. The honeycomb pattern was sporadic and the pores more shallow than the 90 second etched specimen.

X2000

FIGURE 2, inset. Higher magnification of another 120 second etched surface demonstrated tags of irregular shapes and varying lengths.

X4000

mended 60 seconds as the ideal duration for etching the enamel of the occlusal surface for pit and fissure sealing. Davila (1972), using the conditioning solution recommended by Buonocore (1970, 1971), was able to demonstrate penetration of plastic into early carious lesions. Davila et al. (1975), utilizing a gel conditioner, 50% phosphoric acid and an etching time of 120 seconds, demonstrated plastic infiltration into proximal surfaces of molars.

A comparison of 60, 90 and 120 seconds of etching demonstrated that 90 seconds provided a conditioned surface which permitted good infiltration of the plastic material; that is, tag formation was similar to that described as adequate by others investigating plastification of occlusal surfaces (Buonocore, 1955, 1970, 1971; Gwinnett, 1971; Davila, 1972; Davila et al., 1974). Sixty seconds of etching was inadequate in providing satisfactory tag formation. A plausible explanation for the poor etching is that an exposure of the enamel to the gel for 60 seconds was not sufficient to affect demineralization. It appears that the 120 second exposure was excessive and over demineralization of the surface occurred. In this regard it is likely that excursion of the carrier through the interproximal contact areas may have disturbed the topographical characteristics of the etched enamel surface. It is possible that the shallow and irregular pores are also a product of the carrier movement.

Based on these findings, it is concluded that the use of a 50% phosphoric acid gel conditioner and 90 seconds etching time was the most appropriate time for conditioning proximal enamel surfaces of teeth.

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II. Infiltration of a Plastic Material Into Tooth Enamel

(A Comparative Study of Two Technics)

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INTRODUCTION

An etching process is used to condition enamel preparatory to bonding plastic dental materials to the surface. This technic increases wetability, surface area and porosity. Investigators working with plastic materials bonded to tooth enamel have recommended its application of the conditioner with a fine brush so that the weakened fragile surfaces created during etching are not disturbed (Buonocore, 1955, 1970, 1971; Gwinnett, 1971; Davila, 1972; Davila, Buonocore, Greeley and Provenza, 1974). This procedure cannot be used on the less accessible interproximal surfaces.

A new technic which involves the use of a plastic carrier to apply resins to proximal surfaces of teeth has proved successful in removing this limitation (Davila, Sisca, Tinanoff and Provenza, 1975). The effect of the insulating forces on the tooth surface produced by the excursion

of the plastic carrier through the interproximal spaces is still unresolved.

Accordingly, the purpose of this investigation is to compare the carrier technic with the brush technic employing evaluation criteria such as number, size and shape of the tags as determined by scanning electron microscopy.

MATERIALS AND METHODS

Twenty non-carious molar teeth were collected and stored in 10% neutral buffered formalin. Cleaning was accomplished subsequently with flour of pumice and rotary bristle brushes. The teeth were rinsed in distilled water, and divided into experimental and control groups.

Experimental Group

A tooth holder as described by Davila, Sisca, Tinanoff and Provenza (1975) was used for positioning and securing the ten teeth in the experi-

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mental group. The procedures for conditioning the specimen with a gel conditioner* and the application of the self-polymerizing plastic material** by means of a plastic carrier were followed according to Davila et al. (1975). A previously determined duration of 90 seconds during which the tooth surface was exposed to the acid conditioner was employed (Davila and Sisca, 1977).

Control Group

In the control group, ten teeth were etched for 90 seconds with the gel conditioner applied with a cotton pellet. Teeth in this group were held in the operator's hand. They were subsequently rinsed with a water spray and dried with compressed air. The plastic material was applied immediately to the conditioned surface with a fine brush.

Preparation of the teeth for scanning electron microscopy involved sectioning the teeth buccolingually into thirds and immersing the mesial and distal segments in 10% hydrochloric acid until the enamel was completely dissolved and the plastic coating freed as described by Davila et al. (1975). Subsequently, the inner aspect of the plastic coatings were shadowed with gold-palladium and examined on a AMR-1000 Scanning Electron Microscope at 20 KV at varying magnifications (200-1000X).

RESULTS

The techniques described above replicated the etched enamel surface.

The appearance of the surfaces in which the plastic carrier technic was employed was different from those using the brush applicator (Figs. 1 and 2). The topography of the experimental specimens appeared less rugged, the honeycombed areas larger and the tags fewer, more robust and blunter (Fig. 1). The control specimen exhibited more uniform distribution of the honeycombed areas and the tags were longer, more numerous and attenuated (Fig. 2). Areas in which the retentive components were obliterated were less frequently encountered than in the experimental group.

DISCUSSION

Two techniques for applying an acid enamel conditioner to tooth surfaces were investigated in this study. One technique employed cotton pellets for carrying the conditioner to the enamel and a thin brush for applying the plastic material to the conditioned surface. The second technique involved a specially designed plastic carrier for both conditioning and plastification procedures (Davila et al., 1975).

Use of a thin brush facilitates the application of the plastic material to the etched enamel surface. By exerting minimal pressure with the brush, removal or destruction of retentive irregularities created in the surface by the enamel conditioner is reduced.

During the excursion of the plastic carrier through the interproximal space, the etched surface is subjected to insulting forces. It is likely that some of the weakened superficial

* Direction sealant, Tp Laboratories, Inc., LaPorte, Indiana 46350.

** Concise Enamel Bond System, 3M Company Dental Products, 3M Center, St. Paul, Minn. 55101.



FIGURE 1. Scanning electron micrograph of a 90 second etched experimental specimen to which the tooth conditioner and self-polymerizing plastic material had been applied by means of a plastic carrier. Observe areas in which the conditioned enamel has been partially removed probably due to the abrasive forces of the carrier (white arrows). Note that the tags are short and blunt (black arrows).

X1000

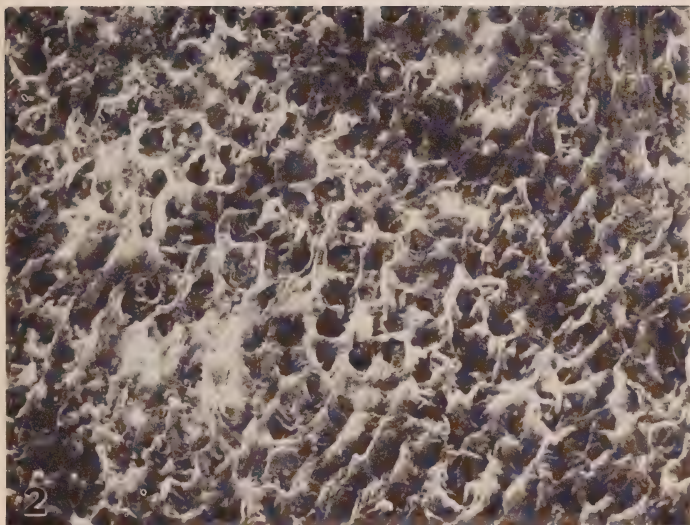


FIGURE 2. Scanning electron micrograph of a 90 second etched control specimen in which the tooth conditioner and resin were applied with a fine brush. The honeycomb pattern is distributed uniformly. The tags are numerous, long and slender.

X200

enamel components were removed. In these areas the excessive removal of etched enamel rendered the surface less irregular and diminished the spaces for tag formation.

It is quite possible that differences in the dimensional and morphological aspects of the tags and their complementary enamel components are a product of the abrasive forces of the carrier in its excursion through the interproximal spaces. The more superficial structures are obliterated. These differences, however, are probably not of sufficient magnitude that they would be a limiting factor in the clinical use of the plastic carrier.

A clinical evaluation is necessary to prove the practicality and effectiveness of this new technique.

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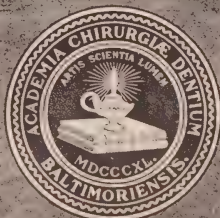
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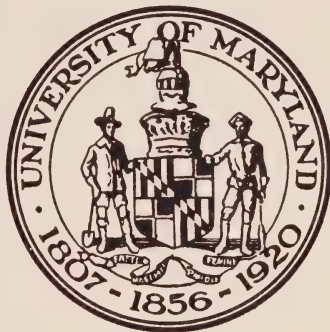
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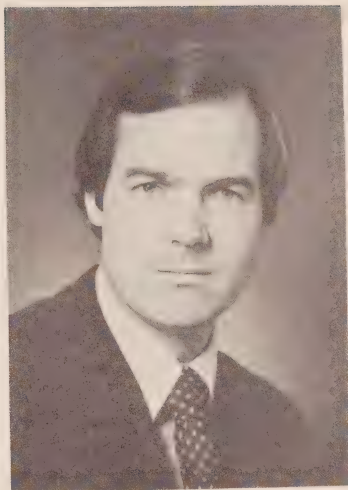
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Dr. Craig joined the Dental School faculty in 1972, and was appointed



Associate Editor of the Journal of the Baltimore College of Dental Surgery in the Fall of 1977.

We welcome Dr. Craig to our ranks.

Lysozyme Activity in Gingival Crevicular Fluid

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INTRODUCTION

Lysozyme is a low molecular weight enzyme which produces lysis of certain bacteria through hydrolytic action on a mucopeptide component of their cell walls (Salton, 1957). The enzyme is widely distributed in tissues and fluids, including gingiva, saliva, and gingival fluid. Tears, nasal, gastric, and intestinal secretions also exhibit strong lysozyme activity, whereas serum has relatively little activity (Brandtzaeg and Mann, 1964).

Increased levels of cationic protein lysozyme have been demonstrated in the serum of monocytic leukemics (Osserman and Lawlor, 1966). Assay of serum and urinary lysozyme is useful to distinguish myeloid and monocytic leukemia. Falchuk (1975) has reported that serum lysozyme levels are significantly elevated in Crohn's disease. Perillie (1968) has confirmed the presence of increased levels of serum and urine lysozyme in sarcoidosis. There is further evidence of increased activity of serum lysozyme in hemodialysis and pretransplant renal failure patients (Daniels, Fukushima, Fish, Lindley, Remmers, Sarles, and Ritzman, 1972).

Lysozyme acts on bacteria by agglutination, preparing them for phagocytosis (Salton, 1957). Lysozyme in gingival fluid may help to reduce the number of bacteria or their virulence (Brandtzaeg, et al., 1964). This may, to some extent, account for the insidious and chronic, rather than acute character of periodontal disease.

Lysozyme may also play an important role in protecting oral tissues by its lytic action on gram positive and certain gram negative organisms (Schultz-Hautdt, 1963). Conversely, some investigators have suggested that damage to oral tissues could occur by endotoxin released from gram negative bacteria lysed by lysozyme (Brandtzaeg, et al., 1964). Likewise, lysozyme of gingival fluid may accelerate the local release of endocellular bacterial enzymes. Hyaluronidase, chondroitin sulfatase, gelatinase, and collagenase of gingival bacteria are mainly endocellular enzymes and are freed during lysis of bacterial cells. All of these endocellular toxins and enzymes may play a role in the etiology or perpetuation of periodontal disease.

During one laboratory study, a paste containing lysozyme was topi-

cally applied on the surface of gingiva of guinea pigs with induced gingivitis. The animals had significantly less signs of gingival inflammation (Morioka, Nishimun, and Matsu-mura, 1970). In another experiment, the addition of lysozyme to the diet of experimental animals did not influence the incidence of caries (Sweeney and Shaw, 1963).

Differences in lysozyme concentrations between subjects with and without gingivitis and periodontitis might indicate the importance of lysozyme in inflammatory reactions (Helderman, 1976). Helderman (1976) has measured the diffusion zone in agar plates from standardized paper point samples and found no significant differences in lysozyme concentrations between noninflamed gingiva and gingivitis in humans. None of his patients had pocket depths greater than four millimeters. Nord (1971) found an apparent trend toward higher lysozyme activity in gingival fluid with generalized gingivitis. Brandtzaeg (1964), collecting gingival fluid by capillary action, found the average lysozyme activity to be approximately 50% higher in the periodontitis group than the gingivitis group. He was unable to collect and compare samples of gingival fluid from clinically healthy gingiva.

A review of these investigations has shown that there was no one study which investigated lysozyme concentrations of clinically normal, gingivitis, and periodontitis patients using the same technique. The purpose of this investigation was to determine lysozyme activity in gingival fluid of human subjects utilizing a standardized methodology and, if present, to evaluate the possible

relationship between periodontal health and disease states.

MATERIAL AND METHODS

Twenty-six subjects with no known systemic disease were classified to Loe's Index (1967) and divided into three groups: clinically normal, gingivitis, and severe periodontitis. The clinically normal group consisted of eleven subjects. The second group included ten subjects with gingivitis and pocket depths from three to six millimeters. The third group consisted of five patients with advanced periodontitis. Gingival fluid was collected around the following teeth in each subject: mesial maxillary right canine, distal maxillary right lateral incisor, mesial maxillary left lateral incisor, and distal maxillary left canine.

Gingival fluid was collected on standardized filter paper strips measuring 2mm by 7mm (Harco Electronics, Ltd.). Each strip was cut at a 45 degree angle and the tip of the strip was inserted into the gingival sulcus or pocket. The technique for the collection of gingival fluid was as follows (Berk and Eisenberg, 1973-4): 1. rinse mouth with water; 2. place one cotton roll on each side of maxilla in the vestibule along premolars; 3. air dry one minute; 4. wait one minute; 5. insert first, second, third, and fourth filter strips at one minute intervals allowing each to remain for a period of four minutes. Fluid was then measured with the Periotron (Harco Electronics, Ltd.). The fluid portion of each strip was cut and placed in a test tube. Enough strips of fluid were collected in the test tube to add up to one hundred

units on the Periotron. The test tubes were then refrigerated.

A modified method of lysozyme assay by Schill and Schumacher (1972) was used. At the time of plating, one cc of distilled water was added to each test tube and agitated for thirty seconds. Two lambda of this solution was placed into a well on the prepared lysozyme plates.

RESULTS

Mean zones of lysis measured 8.9mm in diameter (SD 0.2) for clinically normal subjects; 10.6mm (SD 0.5) for gingivitis subjects; and 13.4mm (SD 0.6) for periodontitis patients. Lysozyme activity was correlated between the concentration of lysozyme and the severity of the disease. There was a significant difference ($p < .05$) of lysozyme activity between the group with periodontitis and the group that was clinically healthy. There were no significant differences ($p > .05$) between healthy and gingivitis groups or gingivitis and periodontitis groups.

DISCUSSION

It is generally agreed that more accurate and objective methods of diagnosing periodontal disease must be developed. It is likewise important to develop methods to determine activity and severity of the disease process. This study and others suggest that the lysozyme concentration in gingival fluid may be useful in diagnosing the severity of the disease process as well as the activity of the disease.

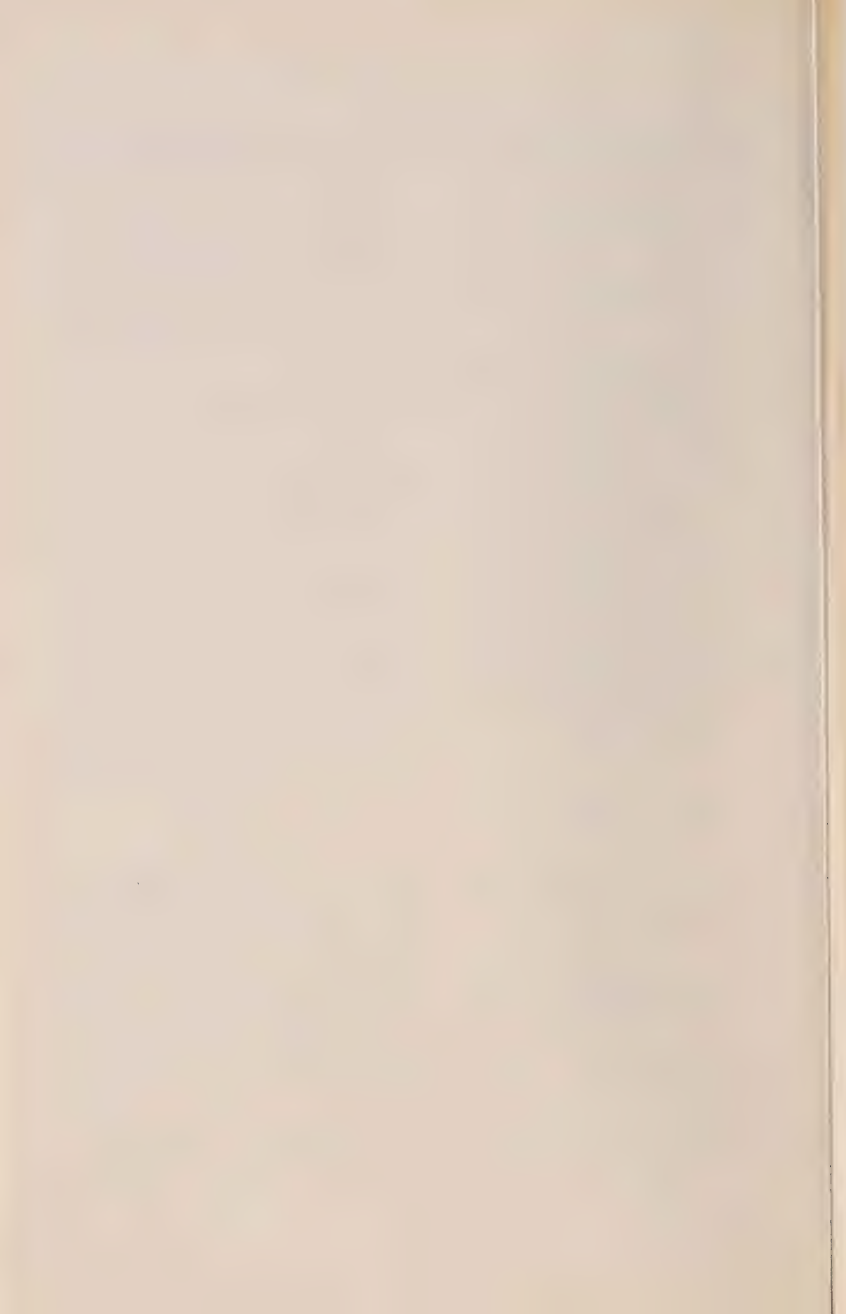
The results of this study are in accord with Helderman (1976) who

found no significant difference in lysozyme concentration between non-inflamed gingiva and gingivitis subjects and Brandtzaeg (1964) who found higher lysozyme activity in periodontitis subjects. One can only speculate as to why there is increased lysozyme activity in patients with periodontitis when compared to patients with clinically healthy gingiva and gingivitis. One possible explanation is that lysozyme is present to serve as a defensive mechanism against the microorganisms within the periodontal pocket. As bacteria are lysed, particularly gram negative bacteria, endotoxin is released which damages the surrounding tissues. The amount of destruction would be in proportion to the amount of lysozyme and gram negative bacteria present. The results of this study support the possible role of lysozyme in the pathogenesis of periodontal disease and suggest that this component may be useful in monitoring the activity of such disease. Additional studies are needed to further evaluate this hypothesis.

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The Intrsubject Reliability of an EMG Integrator-Averager for Bilateral Masseter Activity

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The Intrasubject Reliability of an EMG Integrator-Averager for Bilateral Masseter Activity

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SUMMARY

Electromyography has been utilized in Dentistry with very few means of absolute quantitation. An electronic EMG integrator-averager was developed to provide this quantitation. The intrasubject reproducibility of the device was tested to determine its' reliability. The results conclusively demonstrated that the integrator-averager produced reliable quantitation of muscle activity (based on internal calibration).

INTRODUCTION

Electromyography (EMG) has been used extensively as an investigative aid in dental research over the

past two decades, as evidenced by the reports of Moyers, 1950, Basmajian, 1967, Moller, 1966, Ahlgren, 1967, Griffin and Munro, 1969, Staling and Buxbaum, 1969, and many others. Practical utilization of this valuable source of information however has been restricted by the fact that the majority of the EMG research reports has been either totally qualitative or arbitrarily valued according to the technique used by the investigator. Shpuntoff and Shpuntoff, 1956; Pruzansky, 1960, Ramfjord, 1966 and others have contributed greatly to the concept of the relation of sensorimotor control of jaw musculature to occlusion in spite of the questionable interpretation of qualitative EMG.

The paucity of quantitation coupled with the irreproducibility of EMG tracings has resulted in a minimal clinical use of muscle action potentials as sources of valuable information (Møller, 1970; Kawamura, 1967; Hannam, Matthews and Yemm, 1970; Staling and Gigliotti, 1972; Staling, Fetchero and Vorro, 1976). In the area of myo-oral facial pain and temporomandibular joint dysfunction, clinical use of EMG has contributed within the past five years to firmly establish the primary relation of jaw neuromuscular function to occlusion. In today's social change and complex life style, there is concomitant muscle hyperactivity (Rugh, 1976). Practical use of a biomechanical method that can orient and quantitate muscle activity coincident with mandibular motion, position and occlusion is invaluable to the dental clinician.

The authors felt that sufficient electronic capabilities existed to solve the problem of EMG reproducibility and commissioned the construction of a Dual-channel EMG Integrator-Averager.*

METHOD

The integrator-averager accepts inputs from electrodes. It is fully protected electrically; leakage currents to the patient are limited below 10 microamps under any faulty conditions. Time intervals are selected from a front panel switch. These range from 2 to 24 second intervals. Attenuators (Full-scale/MV controls) set the amplitude sensitivity level of the EMG for monitoring purposes.

The EMG average amplitude level is continuously displayed on a panel meter in millivolts or microvolts. The level of EMG is displayed digitally after a measurement interval, and held until updated by the average of the next interval. The digital display is direct in units of microvolts of EMG activity, average amplitude level.

In order to test the intrasubject reproducibility of the Integrator-Averager muscle runs were done on 15 subjects.

A single run consisted of recording simultaneously average microvolts of muscle activity from the right and left masseters in the following positions: rest, close to contact occlusion, open wide, clench, chew, and swallow.

After proper skin preparation, Beckman surface electrodes (No. 650-951) were placed on the middle of the forehead as a ground, and over the right and left masseter muscles as pick up electrodes. The exact placement position was determined by muscle palpation of the main belly of the superficial muscle mass. Reference electrode placement was achieved by using ear clip electrodes (Beckman No. 214-408). This arrangement provided simultaneous monopolar recordings from both the left and right masseter muscles. (Fig. 1) The electrodes were connected to the EMG Integrator-Averager by means of an electrode lead box. (Fig. 2)

* Engineer, Mark Lomask, Buxco Electronics, Inc.

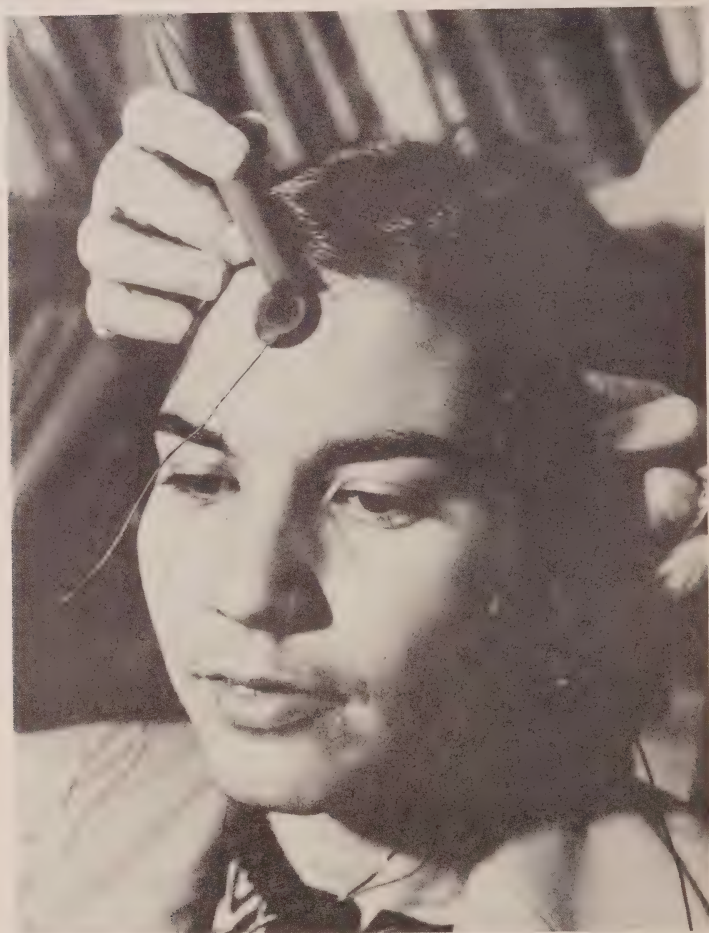


FIGURE 1. Electrode Placement

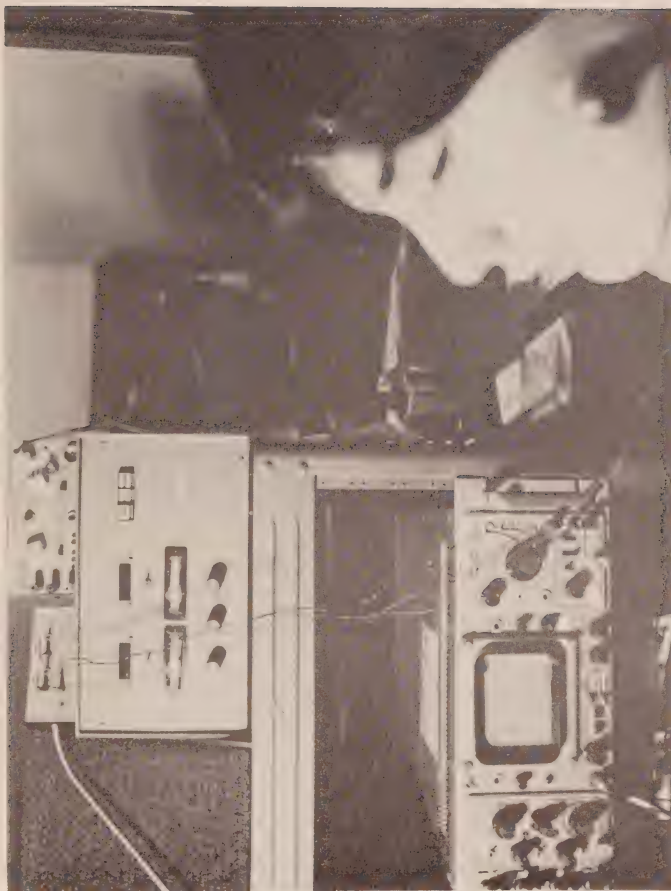


FIGURE 2. Electrode Connection to EMG Input

The device was internally calibrated before each run. The calibrations ranged from 498 microvolts to 501 microvolts. The calibration was reconfirmed during and at the conclusion of each testing session. Readings were obtained every two second time spans at a sensitivity level of 100 or 200 microvolt average amplitude.

Simultaneous recordings of muscle activity, one recording per run, were recorded from the right and left masseter muscles. Recordings were obtained in rest, contact occlusion, wide open, clench, chew and swallow. The electrodes over the muscles were then removed, replaced by another clinician, and then the run was repeated. These runs were repeated five times per session.

The subjects were recalled at varying time intervals until a total of five, five-run sessions had been recorded. This resulted in a total of twenty-five runs per subject divided over five recording sessions.

The recordings were obtained with a 60 cycle filter in line. The subjects were seated in a Dentaleze chair with the head and back positioned such that the occlusal surfaces were approximately parallel with the horizontal.

The subjects were randomly selected. The age range was from 12 years to 58 years. Four of the subjects were male, eleven were female. Twelve subjects had full dentitions. Two had full maxillary dentures occluding with natural teeth, and one was totally edentulous with full maxillary and mandibular dentures.

RESULTS

The average amplitude of muscular activity ranged from a low of five microvolts (recorded while the subject was at rest) to a high of 225 microvolts (recorded while the subject was in the clench position). The standard error of measurement was 22.17. These statistics are presented to indicate the overall level of muscle activity, and to demonstrate that the measurement covered a broad activity range. In all subjects regardless of age or sex, the activity at rest was most stable with a low of five microvolts and a high of 12 microvolts average amplitude. The mean level of activity for the rest position was 7.82 microvolts average amplitude (standard error of measurement = 0.31, inherent "noise" level = five microvolts).

In order to determine the *intra*-subject reliability of the instrument, nonparametric test-retest reliability coefficients were computed upon the data of individual subjects (Anastasi, 1968, Edgington, 1967, Hersen and Barlow, 1976). These statistics were computed upon all possible pairs of test and retest measures across the five recording sessions. The actual performance analyzed was that of the individual participant within a given pair of recording session. Data was computed for each participant and indicated the test-retest reliability of the instrument for that subject (Edgington, 1972). These statistics are presented in Table 1.

Inspection of Table 1 indicates a generally high level of *intra*-subject

TABLE 1
Nonparametric Test-Retest Reliability Coefficients

		Participant														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MUSCLE	RMA	.70	.81	.74	.84	.71	.80	.84	.78	.84	.75	.79	.70	.84	.73	.70
	LMA	.70	.87	.71 ^a	.78	.73	.87	.87	.78	.85	.70	.73	.71	.82	.75	.71
	MA	.70	.84	.73	.81	.72	.84	.86	.78	.85	.72	.76	.70	.83	.74	.70

reliability. Moreover, separate significance tests performed upon the individual MA coefficients indicated that each was statistically reliable ($p .001$). Clearly, both the magnitude and significance of these coefficients establishes the reliability of the instrument for the effective and consistent measurement of the individual subject.

With respect to the group as a whole it should be noted that a separate *inter*-subject reliability analysis of these data lead to identical conclusions. In order to determine the *inter*-subject reliability of the instrument, the data of individual subjects were initially averaged over mandibular position and runs within a given session. This reduction yielded an average measurement score for the RMA and LMA during the five recording sessions. A matrix of reliability coefficients was calculated upon this vector of average measurements and across subjects ($N = 15$). The average test-retest reliability coefficient was computed as .93 and was statistically reliable ($p .001$, Winkler and Hays, 1975). In addition, an autocorrelational analysis and inspection of individual

time series (Chatfield, 1975) failed to reveal the presence of consistent fluctuations in the data.

SUMMARY AND CONCLUSIONS

The level of mandibular muscle activity plays an inherently vital role in *all* aspects of dentistry. This includes but is not limited to research, myo-oral facial pain, and restorative and prosthetic dentistry.

In the recent past devices such as the Myomonitor have been developed in an attempt to diagnose and treat mandibular muscular dysfunction. The quantitation, however, of muscle activity is a vital key essential to determine the tonicity of the mandibular and facial musculature for both investigative and therapeutic purposes (Staling & Buxbaum, 1969).

The results demonstrate that the EMG integrator-analyzer is capable of supplying this need. The intra-subject reproducibility has been established.

During the course of the runs, muscular imbalance was instantly observable in the hyperactivity of

TABLE 2
Average Microvolt Values Integrated over two Second Interval Subject

Jaw Position	Muscle	Session 1					Session 2				
		1	2	3	4	5	1	2	3	4	5
Rest	RMa	8	6	6	7	7	7	6	7	7	7
	LMa	11	8	9	9	10	8	7	8	8	9
Contact Occlusion	RMa	18	10	10	20	15	16	19	16	14	15
	LMa	23	13	19	18	18	15	16	17	27	16
Wide Open	RMa	11	10	9	12	10	12	12	13	12	9
	LMa	12	12	12	15	14	12	14	17	14	11
Clench	RMa	95	120	128	104	107	127	145	122	91	123
	LMa	112	159	190	172	143	184	223	208	146	182
Chew	RMa	31	31	44	27	26	45	33	29	16	22
	LMa	45	45	57	44	38	57	52	48	21	43
Swallow	RMa	39	26	44	42	52	59	26	39	41	57
	LMa	44	52	58	60	69	74	42	58	60	62
Calibrations	RMa	500-500-500					500-500-500				
	LMa	498-498-498					499-498-498				

Sensitivity

the left masseter in subject 6 (Table 2). Occlusal disharmony was determined in this case. In addition, the machine has been used successfully on several occasions to establish vertical dimension on full denture patients.

Further investigations using the apparatus are currently in progress.

The authors feel that a reliable and useful device has been added to the armamentarium of both the dental researcher and clinician.

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Number 6.

Session 3					Session 4					Session 5				
1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
7	8	8	6	7	6	6	8	7	7	7	6	6	7	8
10	9	11	9	9	10	11	9	10	10	10	11	9	9	10
17	20	15	18	16	16	11	18	10	13	18	12	10	19	17
29	23	19	22	22	24	14	21	18	18	17	19	20	19	18
12	10	10	11	12	12	11	9	10	10	10	11	9	10	11
14	15	12	13	12	14	15	13	13	14	12	13	12	15	12
110	122	129	110	108	130	151	137	102	124	101	123	126	119	116
123	146	151	133	127	184	225	214	201	191	120	158	187	163	180
31	33	44	39	37	47	33	30	28	20	28	30	34	31	28
47	49	53	42	40	55	52	50	37	41	45	45	51	46	40
38	32	40	47	51	60	59	39	41	57	27	39	41	47	50
46	48	51	53	59	74	61	58	61	64	48	52	57	61	67
500-500-500					500-500-500					500-500-500				
499-499-499					499-499-499					500-500-500				

200 microvolts

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The Determination of Epithelial Thickness in Selected Oral Sites

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SUMMARY

The thickness of the epithelium was documented at six sites of the oral mucosa. A wide range of epithelial thickness was observed in each specimen, as well as from site to site. The epithelium was thicker at the rete ridge regions than above the dermal papillae.

INTRODUCTION

Currently, there is considerable interest in radiation biology related to the safety of living organisms exposed to irradiation hazards in our environment (O'Riordan and Hunt, 1974). Such a possible hazard may be introduced into the oral cavity by the use of dental porcelains which contain small quantities of radioactive materials, e.g., uranium and rubidium (Moore and MacCulloch, 1974). Exact measurements of epithelial thick-

ness in all parts of the body may be needed to determine the danger of environmental hazards, since the penetration of radioactive substances may effect the normal activity and growth of the germinal layer of the epithelium, as well as, deeper structures.

The normal morphology of the oral mucosa is well recognized and adequate descriptions of microscopic appearance of oral epithelium exist in standard textbooks (Bloom and Fawcett, 1975; Provenza, 1972). The precise measurements of the thickness of oral epithelium are, however, sketchy, conflicting, or lacking (Wentz, Maeir, and Orban, 1952; Binnie and Lehner, 1970).

Certain problems arise when studying epithelial thickness. Measurements may be taken from surface to tip of the rete ridge, or from surface to basal lamina in the area of a dental papilla. Obviously, these measurements would differ widely. Additionally, since oral mucosa is a general

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term, different areas of the oral cavity may be studied and described as oral mucosa. Accordingly, the precise sites examined should be indicated.

This study attempts to provide baseline data for epithelial thickness in areas of the oral mucosa. The values obtained are for thicknesses of epithelium including rete ridges and dermal papillae.

MATERIALS AND METHODS

Twenty human cadavers were used as the source of tissue. Twelve were males and eight were females, three were black and seventeen white. The age range was from 46-87 years. The average age was 67.75 years. A single five millimeter punch specimen was taken from each of the following:

1. *Tip of tongue*: most anterior tip at junction of ventral and dorsal surfaces.
2. *Lateral border of tongue*: at junction of ventral and dorsal surfaces in the premolar region.
3. *Upper lip*: mucosal surface near midline.
4. *Upper lip*: mucosal surface opposite canine area.
5. *Buccal mucosa*: first molar area.
6. *Mucobuccal fold*: mandibular molar area.

Regions 1-5 were selected since they are in contact with teeth or dentures. The mucosa of the mucobuccal fold was chosen for comparison purposes since it is not apposed to teeth.

The tissue (120 specimens) was fixed in ten per cent neutral buffered formalin, hemisected and processed for histological examination. Distor-

tion was minimized by measuring the diameter of the specimen after fixation and again after hemisection. The tissue was cut at three different levels. Step serial sections were prepared and stained with hematoxylin, phloxine and saffron. The sections were independently examined by two microscopists. Only cases with sufficient quantity of well preserved epithelial surface were included. Specimens excluded from the study showed histological evidence of autolysis, surface abrasion or compression. Measurements were made with conventional light microscopy using a calibrated eyepiece micrometer. Each microscopist recorded ten measurements from surface tip of rete ridge for each specimen and ten measurements from surface to basal lamina (in the area of a dermal papilla) between rete ridges. Average figures and ranges expressed in microns were tabulated for the rete ridge area and between ridges for each site and specimen.

RESULTS

The averages and ranges of epithelial thickness for each oral cavity site were compiled using all cases and are presented in Table 1. A wide range of thicknesses was observed at each site with the broadest ranges at the tip and lateral border of the tongue. The latter two areas also had the greatest average thicknesses at the rete ridge region. Similar average thicknesses at the rete ridges were observed for the lip mucosa at the midline and canine area, and the mucobuccal fold. The epithelium above the dermal papilla was thickest at the tip and lateral border of tongue and buccal mucosa. The two lip regions and mucobuccal fold measured had very similar averages and ranges.

TABLE 1
Comparison of Various Sites

	Rete Ridge*		Dermal Papilla*	
	Average	Range	Average	Range
1. Tip of Tongue (summary for 17 cases)	386 μ	94-1125 μ	154 μ	35-562 μ
2. Lateral Border of Tongue (summary for 15 cases)	353	94-1075	147	25-728
3. Midline Lip (summary for 11 cases)	223	83- 940	90	30-333
4. Canine Area Lip (summary for 11 cases)	217	73- 645	96	30-291
5. Buccal Mucosa (summary for 17 cases)	324	125- 915	148	30-385
6. Mucobuccal Fold (summary for 11 cases)	226	40- 475	99	25-294

* 20 measurements were recorded for each case.

DISCUSSION

The data demonstrates a broad range of epithelial thickness in all areas of the oral cavity measured. For this reason average figures based on large numbers of individual measurements are necessary for a valid study. The precise identification of anatomical sites is essential due to the variations in thickness observed from area to area in the oral mucosa. Similar observations were made in the skin where significant variations of epidermal thickness occurs at different body sites (Whitton, 1973). It is also important to note whether the measurements represent the length of rete ridges or the thickness of epithelium between rete ridges (dermal papillae) since the average epithelial thickness of the rete ridges is more than twice that observed at the dermal papillae region of the same site. All factors above were incorporated into the present study.

This study provides data which are in agreement with the previously reported studies (Wentz et al., 1952; Binnie and Lehner, 1970; Whitton 1973). The precise identification of anatomic sites, the measurement techniques, and the large numbers of measurements result in a considerable increase in reliability.

The mean thickness of the epithelium at both the rete ridges and dermal papillae is greater than the maximum penetration range of heavy particles from uranium which is 30 microns (O'Riordan and Hunt, 1974). However, the lower range limits of the epithelial thickness at the dermal papillae in all six regions are at or near 30 microns. Therefore, the maximum penetration range for radioactive particles may extend through some areas of the oral mucosa. Further studies are necessary to determine more precisely the epithelial thickness by using a large

number of human biopsies and the model for measurement employed in the present study. Also, the determination of the nature of intercellular materials and transport, as well as epithelial thickness, is needed to evaluate the potential of the surface epithelium to protect the organism from harmful environmental agents.

CONCLUSION

The present study provides a model for measuring the thickness of the oral epithelium at specific loca-

tions of the oral cavity, noting the structural differences observed at the rete ridges and dermal papillae at each area, as well as from site to site. The epithelia were significantly thicker at the rete ridges than above the dermal papillae.

The ranges and average epithelial thicknesses also varied within as well as among the six areas studied. Therefore, in describing epithelial thickness at the oral cavity, it is necessary to indicate the precise location of the mucosa.

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Articulations of the Medial Pterygoid Plate of the Sphenoid

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ABSTRACT

The medial pterygoid plate of the sphenoid on intact adult human skulls and disarticulated human sphenoid bones articulated with an inferior concha in approximately 87 percent of the cases. Approximately 50 percent of the pre- and postnatal infant skulls in this study possessed an articulation between a pterygoid plate and inferior concha. The medial surface of the medial pterygoid plate was found to be concave rather than flat having become an extension of the inferior and middle meatuses. Fusion of the inferior concha to the floor of the nasal fossa occurred in two of 134 human skulls.

INTRODUCTION

Most textbooks of anatomy list four articulations for the inferior concha (turbinate) namely; maxilla, palatine, lacrimal, and ethmoid bones. Anson (1966), Basmajian (1975), Gardner, *et al.* (1969) Goss (1970), Hollinshead (1968 & 1974), Paff (1973), Romanes (1964), Sicher & DuBrul (1975), Sobotta & Uhlenhuth (1957), Warwick & Williams (1973), Woodburne (1973) are but a few who so list these articulations. Their diagrams of these articulations invariably illustrate the inferior concha extending from the margin of the

piriform aperture at its anterior aspect to the perpendicular plate of the palatine bone at its tapered posterior aspect, attaching by means of a conchal crest.

Close examination of a demonstration skull to determine the articulations of the sphenoid bone revealed that the inferior concha did not terminate on the perpendicular plate of the palatine bone via its conchal crest, but continued completely across the palatine bone and extended onto the medial surface of the medial pterygoid plate of the sphenoid bone. This circumstance posed the question as to the completeness of previously designated articulations for the inferior concha. Thereupon, all skulls and disarticulated sphenoid bones distributed for class use in this department were examined to determine the frequency of articulation of the inferior concha with the medial pterygoid plate of the sphenoid bone.

MATERIALS AND METHODS

The point of termination of the inferior conchae was observed on 134 adult human skulls, representing 268 inferior conchae and medial pterygoid plates. Measurements were taken in a straight line within the inferior meatus from the junction of the inferior concha with the piriform

margin to termination of the conchal crest on either the palatine bone or medial pterygoid plate. Measurement of the floor of the nasal fossa was taken on one side (right) in a straight line between the anterior and posterior nasal spines. Initially, measurements of the floors of the nasal fossae were taken bilaterally. It was observed early that measurements were identical for right and left nasal fossae. Consequently, all subsequent measurements were taken of the right nasal fossae only, assuming that the one measurement was valid for both fossae.

Observations were made on 122 individual disarticulated sphenoid bones (244 medial pterygoid plates) for the presence of an inferior conchal crest on the medial surface of the medial pterygoid plate. On these specimens whenever there was no indication of an articulation with the medial pterygoid plate, it was assumed that the inferior concha articulated with the perpendicular plate of the palatine bone.

Skulls of prenatal (from 7 months) and postnatal (to 7 years) infants were examined. No measurements were attempted on these ten skulls owing to their innate fragility.

Representative specimens were photographically recorded and are presented below. All percentages are rounded to the nearest percent.

RESULTS

Articulation of the inferior concha with the medial pterygoid plate ranged from just touching to establishment of a heavy conchal crest oriented horizontally across most of

the medial pterygoid plate. Figures one and two respectively illustrate these extremes found on the intact skulls, while figures three and four illustrate similar conditions on individual disarticulated sphenoid bones.

Variations in the posterior aspect of the inferior concha included non-tapered, free-ended scroll, tapered, and blunt on the palatine bone and tapered and blunt on the medial pterygoid plate. The inferior concha articulated with 216 of 268 (80%) medial pterygoid plates in 87% of the adult human skulls and 52 of 268 (20%) palatine bones in the remainder.

Measurements of left and right inferior conchae ranged from approximately 32 to 48 mm; left side mean was 42 mm, with a variance of 1.3, and a standard deviation of 3.6; right side mean was 41.4 mm, with a variance of 1.3, and a standard deviation of 3.6. These data indicate only a slight difference between sides.

Articulation of an inferior concha with a medial pterygoid plate occurred in 87 percent of the intact skulls and were equally divided 40 percent each for left and right medial pterygoid plates (Table 1). Evidence of an articulation between an inferior concha and a medial pterygoid plate occurred in 80 percent of the disarticulated sphenoid bones, divided almost equally between left (38%) and right (36%) (Table 1).

A summary of the incidences and combinations of articulations between inferior conchae and palatine bones and medial pterygoid plates of sphenoid bones is presented in Table 1.

TABLE 1

Incidences and combinations of articulations between inferior conchae and palatine bones and medial pterygoid plates found on adult human skulls, disarticulated sphenoid bones, and infant skulls. Parentheses enclose rounded percentages. L = left; R = right; pa = palatine; pt = pterygoid.

	Incidences of Articulations				Combinations of Articulations			
	L pt	R pt	L pa	R pa	Lpt/Rpa	Lpt/Rpt	Lpa/Rpt	Lpa/Rpa
Adult Skulls	108 (40)	108 (40)	26 (10)	26 (10)	9 (7)	99 (74)	9 (7)	17 (13)
Sphenoid	94 (38)	87 (36)	28 (12)	35 (14)	11 (9)	83 (68)	4 (3)	24 (20)
Infant Skulls	3 (15)	3 (15)	7 (35)	7 (35)	2 (20)	1 (10)	2 (20)	5 (50)

Modifications of the medial surface of the medial pterygoid plate for its articulation with the inferior concha were in the form of a conchal crest (the most prevalent form) and/or a conchal tubercle (Figs. 1, 2, & 4).

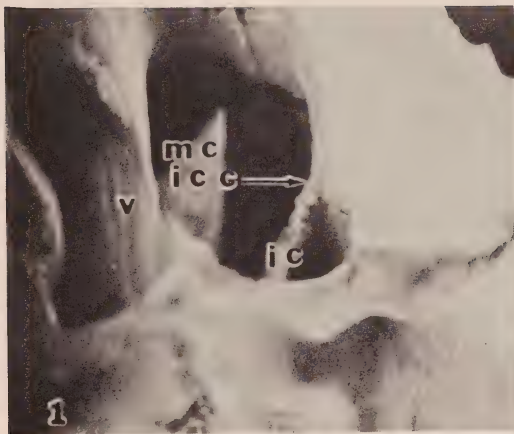
A second crest located inferior to the inferior conchal crest was usually present for articulation with the horizontal plate of the palatine bone (Figs. 3, 6, 7, & 8). A slight concavity existed between these two crests and was confluent with and thus an extension of the inferior meatus. Variation in the depth and extent of the concavity was prevalent (Figs. 2 & 8).

In less than ten percent of the cases a third crest was observed on

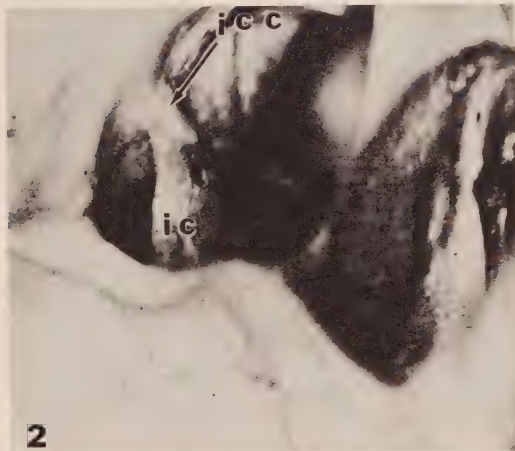
the medial pterygoid plate superior to the inferior conchal crest, articulating with the middle concha of the ethmoid bone (Fig. 9). A second concavity existed between the inferior and middle conchal crests and was confluent with the middle meatus becoming an extension thereof (Figs. 8 & 9).

Mean measurements of the floor of the nasal fossa was 49.9mm, variance was 0.66, and standard deviation was 2.6.

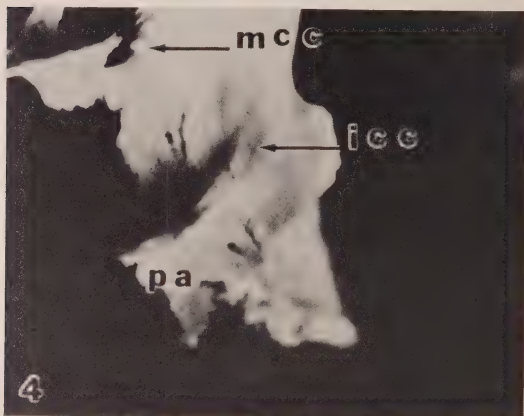
Two examples of fusion between the usually free margin of the inferior concha and the floor of the nasal fossa were observed in these 134 adult human skulls (Fig. 5).



1. Posterior-inferior view of the right medial pterygoid plate on an intact skull illustrating the articulation between a small inferior conchal tubercle and the inferior concha.



2. Posterior-inferior view of the left medial pterygoid plate on an intact skull illustrating the articulation between a large inferior conchal crest and the inferior concha.



4. View of the medial surface of a right medial pterygoid plate illustrating a prominent inferior conchal crest and a middle conchal tubercle. A portion of the palatine bone remains attached to the pterygoid plates.



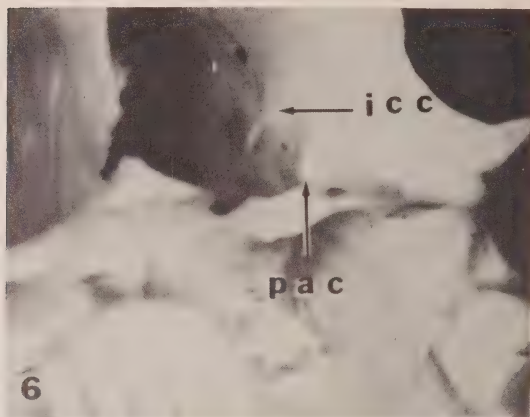
3. View of the medial surface of a right medial pterygoid plate illustrating the inferior conchal crest and the tubercle for articulation with the horizontal plate of the palatine bone.



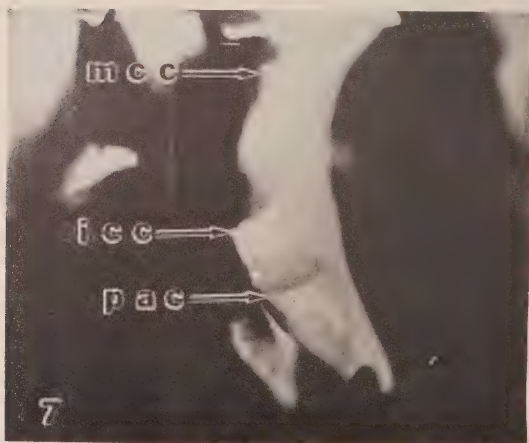
5. View of the left anterior nasal fossa of an intact skull illustrating fusion of the inferior concha with the nasal floor.

KEY TO AND EXPLANATION OF FIGURES:

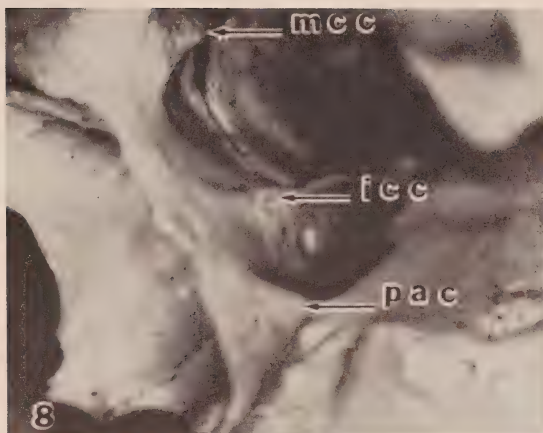
ic—inferior concha	pa—palatine bone
icc—inferior conchal crest	pac—palatine crest
mc—middle concha	s—septum
mcc—middle conchal crest	v—vomer



6. Posterior-inferior view of the right medial pterygoid plate and horizontal plate of the palatine bone of an intact skull illustrating articulation between them via a palatine tubercle on the medial pterygoid plate.



7. View of the medial surface of a right medial pterygoid plate illustrating palatine, inferior conchal, and middle conchal crests.



8. Inferior-medial view of the medial surface of the left medial pterygoid plate of an intact skull illustrating articulations between the middle conchal crest and middle concha, inferior conchal crest and inferior concha, and palatine crest and horizontal plate of the palatine; and illustrating the concave effect on the medial plate.



9. Inferior-medial view of the left medial pterygoid plate of an intact skull illustrating the articulation between the middle conchal crest and the middle concha.

DISCUSSION

On the basis of these observations a fifth bone articulates with the inferior concha, the sphenoid bone via its medial pterygoid plate. An inferior concha articulated with a medial pterygoid plate in 87% of the adult human skulls examined. In these 117 skulls the articulations occurred with equal frequency on right and left sides (40% each).

None of the authors listed above has described any modification of the medial surface of the medial pterygoid plate which they indicated as being flat. The presence of a palatine crest and an inferior conchal crest created a concavity between them. A second concavity was present in those cases where a third crest, the middle conchal, existed. The

presence of two such concavities created a scalloped medial surface rather than a flat surface as previously described. This latter condition was found in less than ten percent of the cases. Thus, the previously described flat medial surface of the medial pterygoid plate does in fact present two or three crests for the palatine, inferior and middle conchae. Significantly longer inferior and middle meatuses are created as a direct result of the increased length of these conchae.

Because of the small sampling of infant skulls firm reliance upon related data presented herein is not recommended.

Fusion of the inferior concha with the floor of the nasal fossa was the only gross abnormality observed in two of 134 adult human skulls.

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**Changes in Serum, Liver, Fat Pad and Thymus
Lipid Levels in Adult Rats Infected with
Plasmodium berghei.**

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ABSTRACT

The effect of parasitemia (*Plasmodium berghei*) on the lipid metabolism of pregnant adult female rats and their progeny was determined in separate tissues: serum, liver, fat pads and thymus. Fatty acids were analyzed by gas-liquid chromatography and spectrophotometric methods were employed for the determinations of triglycerides, phospholipids and cholesterol in the same tissues. The height of parasitemia occurred during delivery and the degree of infection was 7.16% of parasitized erythrocytes. Significant observations were noted in both the adult females and the neonatal thymectomized animals. The infected adult females showed marked increases in triglycerides and phospholipids and significant decreases in cholesterol in all tissues examined. When compared to controls, palmitic acid (16:0, n-Hexadecanoic) was the only fatty acid which showed significant differences. The lipid fraction which demonstrated highly significant differ-

ences in the neonatal thymectomized group were the phospholipids. The significance of these changes with respect to development of hard connective tissue lies in the reciprocal relationship of serum calcium and phosphates.

INTRODUCTION

The present investigation of a dual stress, pregnancy and parasitemia, on the adult and fetal thymus, was part of our studies on the role of thymus in lipid metabolism. Within the past twenty years this originally acclaimed vestigial body has produced multifaceted information linking it to many systems (Luckey, 1973).

In a study of fatty acid changes after lethal body irradiation Chung (1967) reported a decrease in palmitic (16:0; n-Hexadecanoic) and oleic (18:1; cis-9-Octadecenoic and a slight increase in linoleic (18:2; cis, cis-9,12, Octadecadienoic) linolenic (18:3;

9,12,15-Octadecatrienoic) and arachidonic acids (20:4; 5,8,11,14-Eicosatetraenoic). Following the irradiation, the animals received injections of bone marrow, thymus and a combination extract. All three extracts resulted in the increase in the proportions of oleic, linoleic and arachidonic acids in the liver.

Obviously lethal body irradiation is a unique physiological stress of rather uncommon occurrence. Consequently it seemed that perhaps a more mondaine method of stress such as a combined stress of pregnancy and parasitemia merited investigation. The effects of pregnancy on the thymolymphatic system have been described (Nelson et al, 1967). Removal of lymph nodes during pregnancy and the immediate postpartum period revealed a profound depression in lymphopoieses. It is apparent that the thymus humoral factor and its "seeding" ability which is extremely active prenatally and postnatally until puberty, is virtually ineffective if not absent due to the involution of the thymus gland in the young adult. The fact that thymus involution may be accomplished by thyroidectomy was achieved through the administration of quercitin (Masri et al, 1959).

Confinement stress results in thymus involution (Marsh and Rasmussen, 1960). Their studies showed that both the thymus and the spleen were affected over an experimental period of 14-28 days. After 3-7 days adreno-cortical hypertrophy and leucopenia were observed. After the stress was removed, the organs returned to pre-stress status within three weeks. The thymus is dependent on the adrenal cortex for its morphologic and physiologic well being. It

has been proposed that the corticosteroids act as triggers and that the ensuing biochemical events may proceed independently of the hormone (Bellamy et al, 1966). Our studies gained impetus when Tuffrey et al (1969) reported maternal leukocytes in the entire lymphatic system of the fetus.

MATERIALS AND METHODS

A. Selection, grouping and care of animals

Twenty-four female albino rats of the Wistar strain were obtained from the Walter Reed Army Medical Center, Washington, D.C. Twelve randomly selected females were bred and injected with *Plasmodium berghei* sporozoites so that an average of 7.16% parasitemia occurred during delivery. The remaining twelve females composed the control group. All animals were kept in wire bottom cages over wood shavings with Purina Laboratory Chow and water available ad libitum. Pregnant females were transferred to special breeding cages prior to delivery. The twelve litters were randomly divided into the experimental subgroups as listed in Table 2.

B. Thymectomy procedure

The animals were anesthetized with ether in a gallon jar. As soon as all motor reflex activity was abandoned the animal was placed on an operating board and further ether anesthesia as needed was supplied through an inhalant cone. A mid-ventral incision was made through the skin from a point 0.5 cm above the thorax to the level of the fourth rib. Subcutaneous connective tissue

and loose fascia was cut and teased aside and the neck muscles were separated at the mid-line. Prior to the actual opening of the thoracic cavity, two sutures were inserted; one at the level of the first costal cartilages and a second in the musculature between the second and third ribs. Once in place, the sutures were lifted to one side in order to open the chest by cutting the costal cartilages slightly to the left of the sternum. With a pair of blunt forceps the thymus gland was gently loosened from its surrounding connective tissue and consequently removed. The opening was closed immediately with the pre-set sutures and two or three additional sutures closed the neck muscles. All incisions were closed with linen threads. The Thymectomy procedure for neonatal rats was modified after Sjodin et al (1963). In order to effect satisfactory closure of the surgical opening of neonatal thymectomized animals the tissue adhesive methyl-2-cyanoacrylate monomer, kindly supplied by Ethicon Inc., Sommerville, New Jersey was employed. The sham operation was performed in the same manner except that the thymus was left in place.

During the course of the operation the instruments were dipped into seventy percent ethyl alcohol, distilled water and physiological saline to minimize infections.

C. Analytical techniques

The animals were sacrificed by decapitation using Harvard decapitator and bled into a beaker. The blood was centrifuged at 2800 rpm. for thirty minutes and the serum supernatant was removed and transferred into plastic vials. All prepara-

tions were frozen with liquid nitrogen and stored in a freezer at -10°C until the day of analysis. Liver, thymus and fat pad were excised and total organ and tissue weights were recorded. Epididymal fat pads from male animals and adipose tissue of the ovary, ovarian tubes and kidneys of the females were selected for analysis. The tissue was frozen with liquid nitrogen and stored until use.

Fatty acid extraction and methyl ester preparations for gas-liquid chromatography were patterned after Botthcher et al (1959). The amount of tissue to be used was determined through pilot experiments and one gm. of liver, 0.5 gms. of fat pad and one ml. of plasma was found to be adequate for individual analyses. The entire thymus gland was homogenized in a 1:2(V:V) methanol-chloroform solution and 3 ml. aliquots were removed for additional analyses.

The fatty acid methyl esters were chromatographed on a Beckman GC-4 chromatograph fitted with a hydrogen flame detector using ultra pure helium as the carrier gas. Two six foot U-shaped stainless steel columns, $\frac{1}{8}$ inch in diameter and packed with Chromosorb W-DMC, 60-80 mesh, and coated with 15% diethylene glycol succinate were used for the analysis. The Columns were purchased from Applied Science Lab. Inc., State University, Pennsylvania. The columns were operated at a temperature of 180°C and both detector and inlet were maintained at 220°C . The flow rate of the carrier gas was adjusted to eighty ml. per minute, air two hundred ml. per minute.

Fatty acid methyl esters were identified by comparing their relative

retention time to those of known fatty acid methyl esters. Standards were obtained from the Hormel Foundation and run before and at the end of each run. The percentage of fatty acid methyl ester was estimated from peak areas of triangulation.

Serum and tissue triglycerides were determined by the method of Van Handel and Zilversmit (1957). For tissue triglycerides, one ml. of the methanol-chloroform extract prepared for fatty acid analyses was used for liver and thymus and only 0.1 ml. of the fat pad extract was needed for triglyceride determinations.

Serum and tissue phospholipids were analyzed according to the procedure of Zilversmit and Davis (1950). In the case of the liver, 0.2 ml and 0.5 ml for fat pad and thymus of the methanol-chloroform extract were sufficient for these determinations.

The method of Schoenheimer and Sperry (1934) was used to carry out total cholesterol determinations. Tissue cholesterol analyses followed the same pattern except that 0.5 gms. of liver and fat pad were homogenized in the alcohol acetone solution. The entire thymus gland was used.

TABLE 1
Lipid composition of adult parasitized female rats.

	Triglycerides		Phospholipids		Cholesterol	
	Expt'L*	Control	Expt'L	Control	Exptl.	Control
Serum (mg %)	120.2	76.5	24.5	13.2	51.4	75.1
SD	± 15.5	± 7.3	± 7.1	± 1.1	± 3.9	± 2.7
Liver (mg %)	56.1	11.5	30.1	13.5	66.4	90.5
SD	± 8.1	± 0.6	± 3.1	± 0.9	± 6.5	± 4.2
Fat Pad (mg %)	460.9	342.7	52.6	44.6	27.4	37.1
SD	± 34.7	± 27.6	± 3.6	± 2.5	± 4.0	± 2.2
Thymus (mg %)	151.6	139.7	20.1	7.6	24.2	37.1
SD	± 23.9	± 12.7	± 4.0	± 0.8	± 4.0	± 3.7

Exptl.—parasitized pregnant female

control—normal female

SD—Standard deviation

TABLE 2
Lipid composition of tissues of neo-natal thymectomized rat.

		Triglycerides			Phospholipids			Cholesterol		
		E*	S	C	E	S	C	E	S	C
Serum (mg %)	F-2**	39.9	64.8	58.4	7.4	7.2	7.2	80.1	77.3	76.8
	M-2	47.3	71.6	89.6	7.4	6.5	7.4	81.1	75.9	78.2
	F-6	36.9	83.5	70.9	7.3	12.4	11.9	82.8	82.2	81.9
	M-6	49.8	106.9	101.5	7.8	13.8	11.4	80.7	81.6	80.5
Liver (mg/g)	F-2	33.9	7.3	7.9	3.7	9.8	10.5	94.8	75.7	76.9
	M-2	38.7	8.1	7.7	3.8	10.7	10.4	97.9	77.2	84.4
	F-6	58.8	11.2	11.8	3.7	12.8	11.7	113.0	87.9	76.3
	M-6	66.2	12.9	12.3	3.7	11.9	12.5	114.2	85.4	86.3
Fat Pad (mg/g)	F-2	145.1	227.6	221.9	2.2	33.5	32.4	25.1	33.9	35.7
	M-2	152.2	258.2	241.7	2.2	37.8	36.9	26.5	34.9	37.2
	F-6	234.1	342.6	351.8	2.1	41.9	36.6	26.8	43.8	47.2
	M-6	257.9	337.9	342.7	2.0	42.8	43.9	24.1	44.9	44.9

* E—Experimental; S—Sham operated; C—Control.

** F-2, Female, 2-month-old; M-2, Male, 2-month-old; F-6, Female, 6-month-old; M-6, Male, 6-month-old.

TABLE 3
Mean fatty acid composition of serum, liver, fat pad and thymus of parasitized and control adult females.

Fatty Acid	Serum		Liver		Fat Pad		Thymus	
	P*	C	P	C	P	C	P	C
12:0	t	t	t	t	—	—	—	—
12:1	t	t	t	t	—	—	—	—
12:2	1.07	0.90	1.41	1.33	—	—	—	—
14:0	1.13	1.17	1.57	1.29	1.29	0.97	1.38	1.27
14:1	1.06	1.05	1.64	1.53	—	t	t	t
14:2	1.24	1.15	1.73	1.98	—	t	t	t
15:0	t	t	t	t	—	—	t	—
16:0	25.17	17.91	31.49	22.82	30.12	28.76	22.47	16.71
16:1	2.95	2.84	1.94	1.56	3.06	3.57	2.93	3.46
16:2	t	t	t	t	—	—	t	t
17:0	t	t	t	t	—	—	t	t
18:0	11.92	10.79	27.48	27.81	2.12	2.03	7.65	7.55
18:1	25.14	26.08	15.82	15.33	42.83	46.91	39.72	38.73
18:2	32.76	31.88	10.38	18.72	20.71	19.49	15.72	16.19
18:3	3.91	3.56	t	t	—	—	1.47	1.32
20:0	—	—	—	—	—	—	—	—
20:1	—	—	—	—	—	—	—	—
20:4	15.72	16.71	18.61	18.28	—	—	4.67	3.88

* P—parasitized pregnant female.

C—normal female.

TABLE 4
Mean fatty acid composition of serum of neo-natal thymectomized rat.

Fatty Acid	Thymectomized				Sham operated				Control			
	Male		Female		Male		Female		Male		Female	
	2-M	6-M	2-M	6-M	2-M	6-M	2-M	6-M	2-M	6-M	2-M	6-M
12:0	—	—	—	—	t	t	t	t	t	t	t	t
12:1	—	—	—	—	t	t	t	t	t	t	t	t
12:2	—	—	—	—	—	—	—	—	—	—	—	—
14:0	t	t	t	t	—	—	—	—	—	—	—	—
14:1	—	—	—	—	t	1.46	t	1.31	t	1.91	t	1.72
16:0	31.43	29.12	25.86	16.94	17.36	17.49	16.84	18.09	17.80	17.46	17.97	17.97
16:1	t	t	t	1.52	1.25	1.26	1.44	1.37	1.24	1.29	1.08	1.08
16:2	—	—	—	t	t	t	t	t	t	t	t	t
17:0	—	—	—	t	t	t	t	t	t	t	t	t
18:0	32.27	25.15	18.33	6.08	7.09	7.24	6.92	5.73	8.33	7.14	8.41	8.41
18:1	21.16	15.98	15.95	23.14	21.46	23.86	22.15	22.14	22.47	21.88	23.18	23.18
18:2	5.73	15.14	18.47	28.79	25.91	31.42	29.68	29.87	25.84	30.09	28.32	28.32
18:3	—	—	—	1.82	1.57	1.66	1.26	1.29	1.81	1.27	1.04	1.04
20:0	—	—	—	—	—	—	—	—	—	—	—	—
20:1	—	—	—	—	—	—	—	—	—	—	—	—
20:4	t	10.73	21.94	10.47	9.82	11.89	9.21	11.42	9.62	12.57	8.62	8.62

TABLE 5
Mean fatty acid composition of liver of neo-natal thymectomized rat.

Fatty Acid	Thymectomized						Sham operated						Control					
	Male			Female			Male			Female			Male			Female		
	2-M	6-M	2-M	2-M	6-M	2-M	2-M	6-M	2-M	2-M	6-M	2-M	2-M	6-M	2-M	2-M	6-M	2-M
12:0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12:1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
12:2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14:0	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
14:1	t	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
14:2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16:0	29.04	30.97	30.18	25.27	23.48	24.84	24.84	25.97	24.22	22.76	24.73	24.64	24.73	22.76	24.73	24.64	24.73	24.64
16:1	t	t	t	1.50	t	1.82	1.82	t	1.31	t	1.72	t	1.31	t	1.72	t	1.72	t
16:2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
17:0	t	t	t	1.01	t	1.24	1.24	t	1.09	t	1.27	t	1.09	t	1.27	t	1.27	t
18:0	32.08	29.14	31.68	12.78	22.19	12.96	12.96	21.82	13.18	24.34	13.91	22.52	13.18	24.34	13.91	22.52	13.91	22.52
18:1	10.17	10.59	9.73	16.39	11.47	18.81	18.81	11.95	17.27	13.80	17.25	14.08	17.27	13.80	17.25	14.08	17.25	14.08
18:2	15.93	15.08	14.88	21.04	18.92	24.60	24.60	18.74	10.48	10.76	23.21	18.73	10.48	10.76	23.21	18.73	23.21	18.73
18:3	—	—	—	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t
20:0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20:1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20:4	10.46	9.76	11.17	10.18	10.86	9.27	9.27	16.91	11.88	11.73	11.09	16.49	11.88	11.73	11.09	16.49	11.09	16.49

OBSERVATIONS

The serum, liver and fat pad sample of each animal and the thymus of all non-thymectomized animals were analyzed for the triglyceride, total lipid soluble phosphorus and the total cholesterol concentration. The total cholesterol represented the free cholesterol plus the esterified cholesterol. The serum values for these determinations are expressed in mg. per 100 ml of serum (mg. %). Data obtained from all other tissues are expressed in mg. per gm. wt of tissue analyzed. The triglyceride, phospholipid and cholesterol analyses were performed in triplicate per animal tissue. Data are reported in Tables 1-6. Tables 3-6 list only the major fatty acids although data is available for others which are not included here. All figures listed are percentages of the total fatty acid concentration per tissue. Statistical significance was set at the 5% level based on a planned comparison of means.

Comparison of the adult parasitized and control animals indicates substantial increases in both triglycerides and phospholipids in all examined tissues of the parasitized females. Cholesterol however showed a significant decrease. It is most probable that cholesterol is utilized for increased synthesis of the adrenocortical and ovarian steroids (Table 1 and 2). Table 3 lists the fatty acid composition and it appears that only the saturated fatty acid, palmitic acid, (16:0) showed a significant increase in the parasitized females.

Conclusions drawn from the data for neonatal thymectomized animals are less defined. The differences observed are evident in both the 2 and 6 month groups suggesting that

changes in lipid metabolism are to be of long duration. Serum and fat pad triglycerides decreased in these groups but a significant increase was observed in the liver. Phospholipids showed a general decrease in all tissues. Cholesterol levels in the serum of experimental animals were comparable to those of the sham operated and controls. However, higher levels of cholesterol in the liver and lower in the fat pads of experimental animals were observed as compared with the other groups. No significant changes were observed in the fat pads. There is an increase in palmitic (16:0) and stearic acid (18:0) in the serum and liver.

DISCUSSION

The fact that genetic differences among rat strains may show reverse effects on serum and tissue lipids was pointed out by Boyd (1942), Broadhurst (1960), Feuer (1963) and Skanse (1959). Therefore, in establishing the responses of the Wistar strain under multiple experimental conditions, a better evaluation could be made of the triglyceride, phospholipid, cholesterol and fatty acid levels of the various tissues.

The Wistar albino rat was chosen for these experiments since thymectomy does not produce in these animals the usual wasting syndrome described by Law (1964). Some of the most common symptoms of animals thymectomized between one and four weeks of age are weight loss, lethargy, dorsal kyphosis, peri-orbital and facial edema, rough unkempt fur, posterior cervical alopecia, unsteady gait, anemia, slight reticulocytosis, thrombocytopenia, mild leukopenia and striking depletion of body fat as well as a marked

reduction in spleen size. Manning (1959) showed conclusively that the body weight of the Wistar strain rat is not affected by thymectomy and the general impression was that these animals were healthy and grew as well as the intact animals. These observations were supported by recorded body and organ weights. The greatest mortality rate was found among adrenalectomized and combined thymectomized and adrenalectomized animals. It appears that death was due to adrenal insufficiency since none of the animals died a few days after surgery.

The study on the parasitized female rat was introduced to not only show the effect of parasitemia on lipid metabolism but also to test the significance of the maternal well-being on the development of the thymus in her offspring under these conditions. Brand and Mercado (1958) demonstrated a reduced glycogen content and an impaired glycogen synthesizing power of the liver in rats infected with *Plasmodium berghei*. The administration of certain steroids partially reversed the lowered glycogen content and impaired glycogen synthesizing power of the livers of these rats. Glycogen free areas became infiltrated with fats in animals infected with *Plasmodium berghei*. It has also been demonstrated that similar fatty infiltrations also occur in human malaria. Kaplan and Chaikoff (1936) have shown that while the typical antagonism between fat and glycogen occurs often in abnormal nutritional states, diabetes, or as a result of toxic influences, large amounts of fat do not necessarily interfere with the capacity of the liver cells to store glycogen as long as these cells are not injured by toxic substances. Consequently, *Plasmodium berghei* may not be primarily

responsible and it is probably that adrenal dysfunction is involved to a greater extent than expected.

The maternal influences of suckling survival and the development of lymphoreticular organs were examined by Albert et al (1965, 1966). By comparing offspring born to primiparas of increasing age, they found that maternal aging decreased body weight, survival, mitotic activity and weight of the lymphoreticular organs of their offspring. Their data also indicated that in tumor bearing dams small decreases occurred in the body weights of their female and male offspring, but an increase in the mitotic activity of the lymph nodes of only female offspring was detected. Therefore, survival and development of the lymphoreticular system of progenies were influenced by the maternal physiology. It is possible then that the decreases in infant survival noticed by these authors occurred primarily due to increasing maternal age. Albert (1965) also postulated that maternal age affected the development of the thymus in the offspring.

Since our parasitized adults and the neonatal thymectomized progenies basically constituted two separate experimental groups, it is perhaps advisable to treat the raw data separately for each group and then infer possible congenital metabolic errors due to the maternal physiological status quo. The increase in triglycerides seen in serum, liver and thymus of the adults are not too surprising if one considers the lipid mobilizing effect of stress plus the effect of any stress on thymic involution which basically involves the deposition of lipid in this gland. The high triglyceride content of adipose tissue was unexpected and can pos-

sibly be attributed to imbalances in both lipid and carbohydrate metabolism. One explanation may be decreased activity of adipose tissue lipase which over-shadows the lipid mobilizing effects of stress released catecholamines and ACTH. As was pointed out earlier, rats infected with *Plasmodium berghei* showed an impaired glycogen synthesizing ability which would lead to hyperglycemia with increased releases of insulin. Insulin has been shown repeatedly to foster lipid synthesis. In addition, the effects of ovarian and placental steroids on lipid metabolism are relatively ill defined.

The rise in phospholipids of the parasitized adults is secondary to the increases in triglycerides. The liver will pick up extra free fatty acids and convert them to phospholipids and slightly later some will be changed to cholesterol esters (Mead and Fillerup, 1957). Although this would predict an increase in cholesterol, our observations do not concur. Again, if one takes into consideration the hyperglycemia induced by *Plasmodium berghei* and its resultant effects on the adrenal steroids one can justify this cholesterol decrease due to increased steroid synthesis.

The actual fatty acid composition remained remarkably constant. The unesterified fatty acids are transported as a complex with serum albumin entering adipose and other tissue cells. These fatty acids have many fates: utilization for energy; storage as triglycerides in adipose tissue cells; esterification with cholesterol or perhaps other sterols; or, synthetic building blocks for phospholipids. Diet studies conducted by Hirsch (1962) led to the proposal that adipose tissue may contain two compartments; one which represents

approximately 90% of the lipid content and is chemically inert and the other, about 10% which shows a rapid turnover. This could substantiate the tremendous increase in fat pad triglycerides as well and the observed stability of fatty acid levels.

The results for the triglyceride, phospholipid and cholesterol levels for the neonatal thymectomized animals are summarized in Table 2 and their fatty acid values are listed in Tables 4-6. Scanning the mean mg. % values it is readily detectable that these values are significantly different from the sham operated and control groups. Furthermore, these changes are not of short duration since the six month groups reflected them as well. Consequently the maternal effects on the development of the lymphoreticular system seem equally as effective as the thymectomies. Fat pad and serum triglycerides are decreased but a significant increase is seen in the liver. It appears that the metabolic error resides in the liver since it is difficult to reconcile the fact that such a great quantity of lipid derived energy is needed for the growth process. The decreases in liver phospholipids also shows that this conversion mechanism lost much of its activity. Serum cholesterol appeared stable with only minor decreases in fat pads. Again, liver cholesterol showed a significant increase. Unfortunately this study was not extensive enough to detect the error, i.e., are we dealing with increased synthesis or decreased catabolic reactions. The desaturation capacity of the liver seemed functional since no significant differences were apparent. Parasitemia may have limited this hepatic ability somewhat in the adults where noticeable differences were observed with respect to increases in palmitic acid.

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The Evaluation of a Slide Tape Presentation for the Orientation of Students to Clinical Screening

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The Evaluation of a Slide Tape Presentation for the Orientation of Students to Clinical Screening

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ABSTRACT

Synchronized slide-tape presentations involving clinical techniques are in wide use in dental education. There have been few reports verifying their reliability and validity prior to their clinical application. This project tests the validity of a slide tape program in the clinical area of student screening of prospective patients for the comprehensive care clinics. The results indicate that students who were exposed to the slide tape program came to their clinical assignment with significantly greater understanding of all of the aspects of clinical screening. Moreover these students performed better clinically thereby enhancing the overall efficiency and effectiveness of the clinical screening program.

INTRODUCTION

The utilization of synchronized slide-tape presentations has rapidly gained acceptance as a means of instruction in dental education at the University of Maryland Dental School. A great deal of data asserts

the effectiveness of this instructional medium in the realm of didactic information. Relatively little research has been done to verify its usefulness in teaching clinical technique, even though many clinical productions exist. Clinical procedures in dentistry are generally of a diverse nature and therefore difficult to present in a precise definitive method. In some aspects of the dental clinic, techniques may be broken down into definitive steps that do not present a great deal of variation. The new comprehensive system of screening of patients as teaching cases at the Dental School represents such an area. In this study the effectiveness of instructing screening techniques to students by synchronized slide tapes will be evaluated.

Each new patient who applies for comprehensive care at the Dental School must first be screened by the Oral Diagnosis Department. This standardized screening procedure insures an accurate determination of the patients needs and accurately matches these patients with the educational experiences needed by the undergraduate students. Since efficiency and effectiveness of data gathering are of utmost importance in determining student needs, a definitive routine must be followed, particularly since a computer method of matching patient treatment and student experiences is

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planned for the future. Third year dental students are assigned to perform new patient screening under the supervision of an oral diagnosis instructor. Due to the great number of patient applications, speed of screening is a goal of the program; hence thorough knowledge of the forms, necessary materials, and the steps involved in a comprehensive screening is mandatory for all students who participate in screening.

PROCEDURE

With these factors in mind the Oral Diagnosis Department contacted the Office of Educational Instructional Resources to discuss the possibility of a program to teach clinical screening. A synchronized slide tape was selected as the mode of instruction. Although mass screening is not within the normal job description of private dental practitioners, all of the techniques and principles involved in screening apply to private practice. The script for the slide tape was developed by the authors with the assistance of the Department of Educational and Instructional resources. Since these third year students to date had received little clinical experience, care was taken to develop a slide tape to cover each step in the thorough screening of patients. An armamentarium was shown and described as well as the ten diagnostic steps necessary to qualitatively and quantitatively classify the patients. The ten steps were described in detail in the tape along with clinical photographs to demonstrate each particular technique. A pre and post test design was selected to evaluate the slide tape as well as a clinical evaluation of student screening technique.

METHOD

After editing of the manuscript and final review of the slides, developmental testing was initiated using 30 students of the third year class. All testing was administered by the authors in the Oral Diagnosis Screening Clinic. The computer student assignment schedule was used to determine the thirty student sample. The students are assigned to screening in groups of three for three days each. The students had no previous experience in screening new clinic patients. A control group of ten students was selected from the sample for a pre-test. Every third student was randomly placed in the control group. The pre-test was administered to students in the Independent Learning Center in an unproctored fashion. The test consisted of three questions asking the student to describe various aspects of what is involved in screening. Twenty students were used in the experimental group. These students also took the pre-test. The pre-test was scored using one point for each correct answer with a maximum of twenty five points. The experimental group of students then independently viewed the slide tape presentation in the ILC. A post test was then administered to all students which covered the same material as the pre-test. Statistical analysis for variance using the T-test was done to determine differences between the control and experimental groups. Following this testing both groups were assigned to the clinic screening area for clinical technique evaluation.

The criteria for clinical evaluation of mastering the screening techniques was based on an evaluation by a single faculty member during each student's three day assignment in the screening clinic. This evaluation was based on areas

of student confusion. A scale was developed to evaluate the content of the questions and their frequency, as well as accuracy of following the clinical steps involved in the screening. The evaluator did not know which students were in the control and experimental groups.

RESULTS

The results of pre and post tests are listed in Table I. The individual scores for the control and the test groups were compiled to arrive at a mean group score for each group. On the pre-test evaluation the mean scores were 12.5 (control) and 12.2 (experimental), which indicates that the knowledge level of the students prior to slide tape exposure was equal. The post test scores indicate significant change ($p < .001$) in performance on the didactic portion of the study for the experimental group. The control group did not show significant improvement ($p > .25$) on the didactic portion.

Table II lists the trends of the student groups relative to their clinical evaluation during their screening assignment. The control group was essentially unable to perform the clinical techniques without initial explanation of procedures to follow. Since both groups were comprised of junior students, anxious to perform well clinically, the accuracy of the two groups was reasonably comparable although subtle differences were apparent in favor of the experimental group. These students were able to direct their efforts to the more important areas of screening evaluation with less time spent on the actual technique.

DISCUSSION AND CONCLUSIONS

The results indicate that the ex-

perimental group improved in performance on the didactic portion of the post test. The highly significant improvement reaffirms the effectiveness of slide-tape programs for introducing material to students. The subjective clinical evaluation confirmed that students who viewed the slide tape also performed better clinically, spending less time and effort on technique and more time on accurate screening evaluation. Efficiency of clinical time and technique proved to be the areas of greatest improvement for the experimental group. The experimental group overall asked fewer questions on technique of screening. Accuracy of screening evaluation was also higher for the experimental group since less time was needed to be spent on developing and/or clarifying clinical technique. Overall efficiency and effectiveness of screening was therefore improved by the slide tape exposure to the clinical technique of screening.

This brief study indicates that independent study of clinical procedures presented by slide tapes prior to clinical exposure in a given area can be extremely useful in presenting the didactic portion of clinical technique and in increasing efficiency and effectiveness of student performance. In the area of screening this technique of presentation has been very valuable in developing our goal of accurate screening of new dental clinic patients and thereby has enhanced the implementation of the computerized student assignment program. In this program we plan to match patient treatment needs with anticipated student needs and experiences.

By using the computer feedback system as students progress through

treatment planning and actual treatment of the patients, a data bank will be established that can be compared with the screening information. As the implementation of this system is fully accomplished, further study and evaluation of the accuracy of this comprehensive screening system will be possible and necessary.

TABLE I

Pre and Post Test Results

Control Group (n=10)

	Pre-Test	Post-Test
mean	12.5	14
range	3-20/25	3-22/25
p>.25		

Experimental Group (n=20)

	Pre-Test	Post-Test
mean	12.2	20.8
range	4-18/25	10-25/25
p<.001		

TABLE II

Trends in Clinical Evaluation

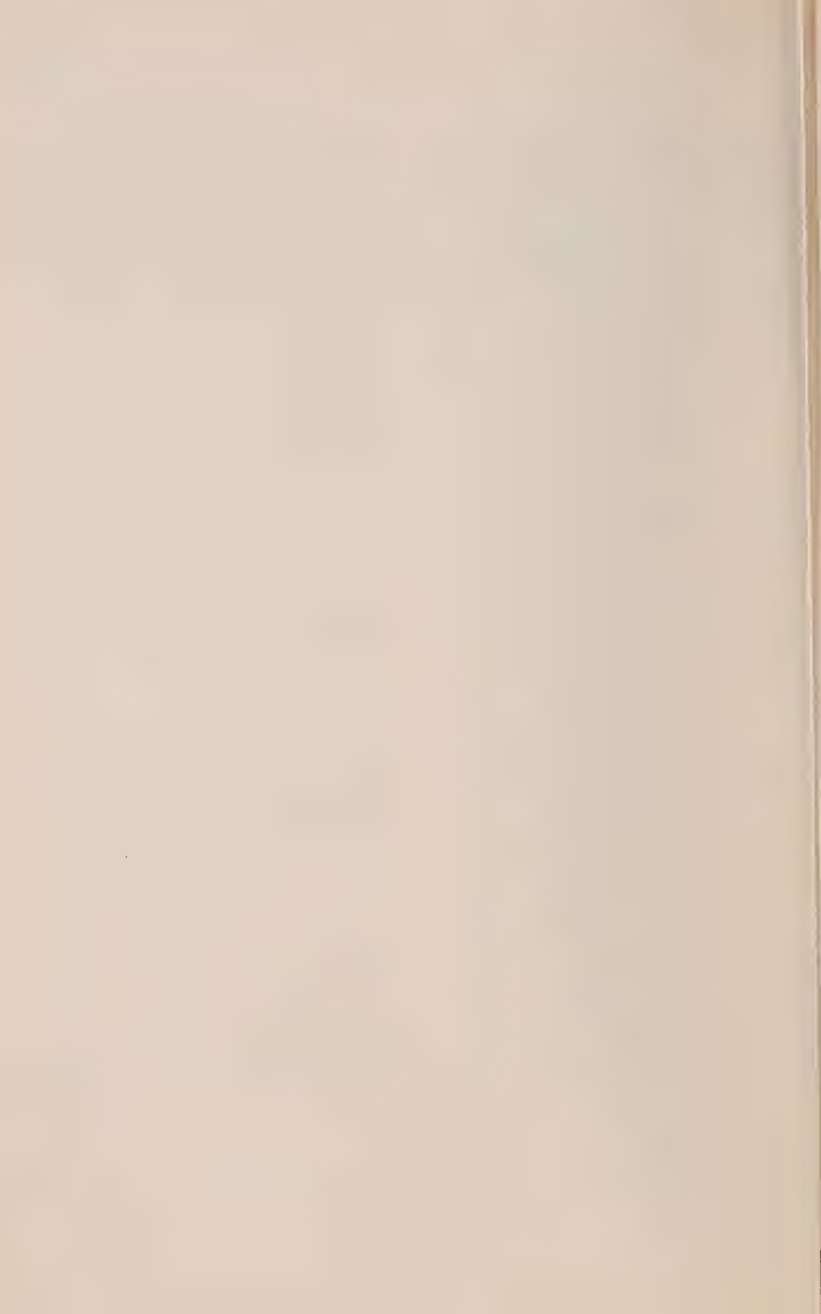
	Control Group	Experimental Group
Frequency of questions	very frequent	rare
Content of questions	basic	case related
Omission of armementarium	occasional	rare
Omission of steps	frequent	rare
Accuracy of Clinical Technique	fair	good

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DEVELOPING UNTAPPED RESOURCES

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DEVELOPING UNTAPPED RESOURCES

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SUMMARY

The Faculty Council Committee on Library and Publications and the Department of Educational and Instructional Resources have established a cross-index file of the departmental collections throughout the School. The rationale and procedures for this cross-indexing system, which is designed to *conserve resources*, is presented here.

INTRODUCTION

Tight budgets can lead to many innovations. Not only can they provide a ruthless means of determining essentials, they can also bring about the demise of outmoded ways of thinking and give birth to new ideas. When money allocated to library budgets began "drying up", the concept of a self-contained, self-sufficient unit for sustaining and

maintaining the research activities of students and faculty was no longer viable. In order to meet the continuing demand of these individuals, however, librarians developed many variations on the theme of resource sharing. One such variation took place at the Baltimore College of Dental Surgery, Dental School, University of Maryland at Baltimore where it was decided to develop the untapped resources within the Dental School itself through the implementation of a cross-indexing file for departmental collections. Although the example is that of a health science institution, the cross-indexing system can be adapted by any school divided into departmental units and can include both print and non-print media. Such a system is a simple, though often overlooked, way to gain or extend accessibility to a wide range of material.

RATIONALE

As in most educational institutions, departments in the Dental School are allocated a discretionary budget, some of which is spent on books and journals to satisfy the references or teaching needs of its members. Unfortunately, few people outside of the department have knowledge of the existence or extent of these departmental collections. As a result, there is unnecessary duplica-

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tion of some material, while other information needs go unsatisfied.

The Dental School houses an Independent Learning Center (ILC) with over 1400 titles in a variety of formats, including slide/tapes, videocassettes, manuals, film loops, etc. It also has a journal collection made up primarily of state dental association publications with several of the more scholarly journals like the *Journal of Dental Research* and *Dental Clinics of North America*, and, of course, the *Journal of the American Dental Association*. The major journal and monograph collections on campus are located at the Health Sciences Library. The Dental School curriculum, however, does not provide for a great deal of free time to take advantage of this relatively remote facility and students often look to the ILC to satisfy their information needs.

PROCEDURES

As part of its obligation to the School, the Department of Educational and Instructional Resources, in cooperation with the Faculty Council Committee on Library and Publications, set out to determine the feasibility of sharing the resources within the School. First, it was necessary to determine the extent of the collections to find out if establishing a cross-index file would be worth the effort. The data accumulated from the survey of resources indicated the need for establishing a cross-indexing system (Figure 1).

To ensure active participation by all departments, Dr. Errol L. Reese, Dean of the Baltimore College of Dental Surgery, Dental School, University of Maryland at Baltimore requested the support of the Executive Committee, composed of all department chairmen within the

Dental School, for the implementation of the file. The assistance of the Director of the Health Sciences Library was also sought and received. When representatives of the Committee met with the individual department chairmen to discuss the project, ground rules were established and agreed upon. First, responsibility for checking out departmental materials remained with the department. Second, the privilege of checking out materials could be refused anyone who had demonstrated irresponsibility in the past. Third, the department reserved the right to restrict the use of some materials. Fourth, departmental personnel would not be asked to take on any additional work in compiling the file. Instead, students were used to gather data and the Dental Faculty Wives volunteered to help with the typing. The media specialist/librarian in the ILC supervised the project. Finally, the cross-index file was to be integrated with the present ILC card catalog.

It was decided that access would be through main author, title, and department. No attempt was made at subject cataloging, since the very fact that a book was selected by a particular department such as Biochemistry or Pharmacology would provide access enough for purposes of this project. Also, the format to be used for the catalog cards was designed to be compatible with those already in the file.

Once all these decisions had been made, the media specialist/librarian trained the students selected to assist in this project in the techniques for gathering the cross-indexing information. The form (Figure 2) identified the relevant information students needed to obtain from each book or

journal. During the initial phase of the project, careful supervision was necessary to ensure the forms were being filled out correctly. As a further control, only the collection of one department was worked with at a time. Once the data was gathered, it was transferred to the cards (Figure 3) to be placed in the ILC card catalog.

CONCLUSION

Periodic review of new materials in the departments is required to keep the index up-to-date. The system also allows the private collections of individual faculty members to be added should they be interested in making them available. The cards for these private collections could easily be removed from the file, when, or if, the instructor leaves, by finding cards filed under the appropriate department which bear his/her name and taking out the three cards for each entry.

In order to successfully accomplish this in-house resource sharing, the following things must be kept in mind. First, clarify your intent to all those concerned and gain their support and approval. Then, design your tools carefully, supervise the work, and establish a system of maintenance.

Cross-indexing at the Dental School has aroused the interest of other schools on the University of Maryland at Baltimore campus and it may be adopted by them. It is essential to realize that such a system, though fairly easy to maintain once instituted, does require continued clerical support. The favorable reaction of the students to the concept and the support given by faculty and department personnel in its development would seem to make such a commitment worthwhile.

SURVEY RESULTS OF DEPARTMENTAL HOLDINGS

Baltimore College of Dental Surgery
Dental School
University of Maryland at Baltimore

Number of Faculty	178
Number of Students	673
Number of Volumes	2,867
Number of Journals	153
Number of Square Feet Devoted to Library Storage	1,199
Full or Part-time Librarians	None
Professional Librarians	None
Number of Hours Open Per Week	40
Annual Expenditures	\$5,960

FIGURE 1.

Author _____

Title _____

Edition _____

Place of Publication _____

Publisher _____

Date _____

Number of Pages _____

Department _____

Location (Room) _____

Contact _____

FIGURE 2. Data-gathering form used by students working with departmental collections.

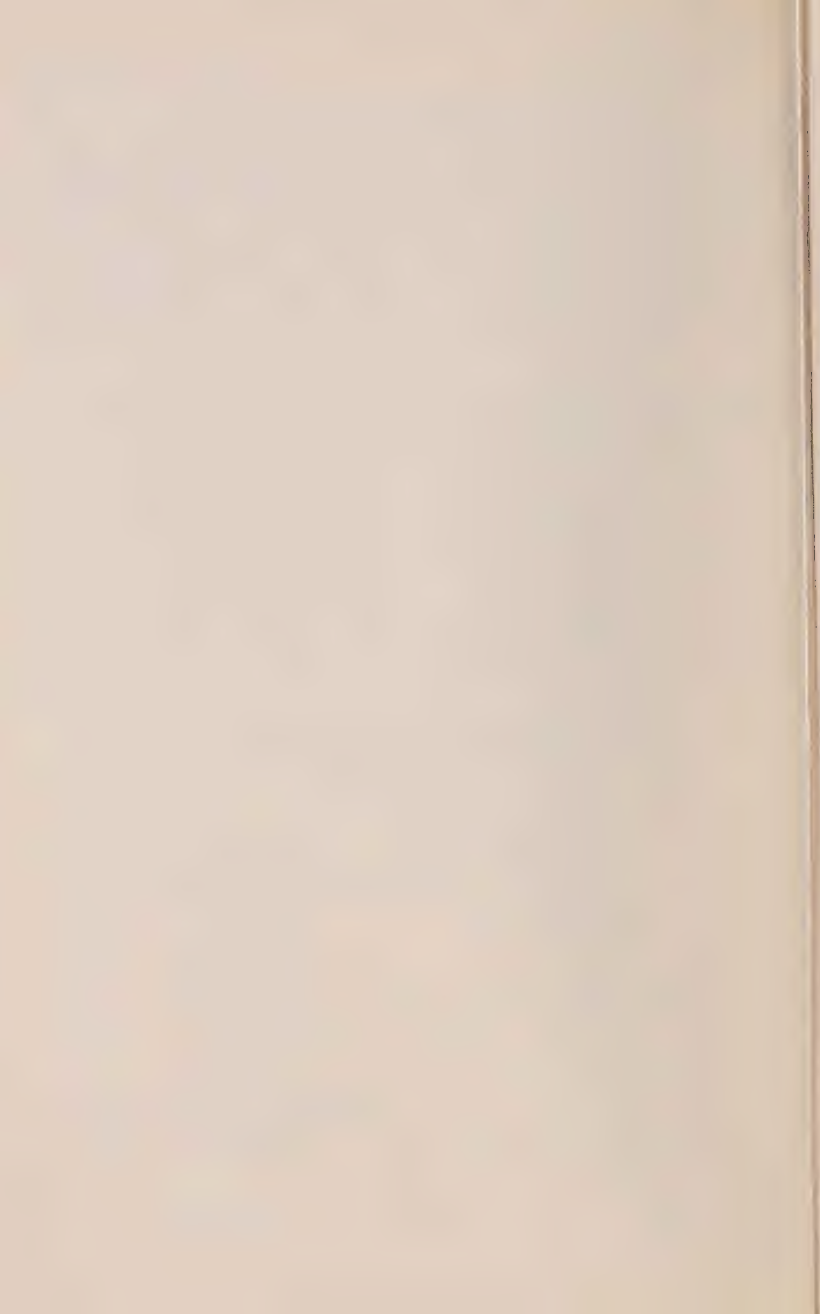
Burns, George W.
The science of genetics: an introduction to
heredity, by George W. Burns. New York,
MacMillan, 1969.
399 pp.

Location → Anatomy Department

Contact Dr. Meszler

↑
Indicates Private Collection

FIGURE 3. Finished catalog card ready for the cross-index file.



Surface and Thin Section Ultrastructure
of
Fusobacterium polymorphum

CHARLES E. HAWLEY*
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JAMES R. MONGIELLO
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Surface and Thin Section Ultrastructure of *Fusobacterium polymorphum*

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SUMMARY

Fusobacterium polymorphum, ATCC 10953, was examined by transmission electron microscopy after growth in broth. When negatively stained with phosphotungstic acid, the organisms displayed the typical convoluted gram negative surface morphology and extreme variability in cell length. The characteristic three layered morphology of the gram negative cell envelope was demonstrated in ultra-thin sections of these oral anaerobic bacteria. Using ruthenium red/osmium tetroxide fixation, the organisms also displayed a surface layer of dense polysaccharide granules. In some sections, an outpouching of the outer membrane was observed which enclosed an enlarged area of the periplasmic space. This sac-like structure corresponded to the spherical polar appendages that were occasionally seen in negatively stained material. It is proposed that the outer membrane lined sac and

the surface polysaccharide coat of this organisms may play important roles in its colonization of the gingival crevice and in its pathogenic potential against the human periodontium.

INTRODUCTION

Fusobacterium polymorphum, a gram negative filamentous bacterium belonging to the family *Bacteroidaceae*, has been shown to increase numerically in cases of advanced periodontitis (Sabiston and Grigsby, 1972; van Palenstein Helderma, 1975). Early reports describing the oral *Bacteroidaceae* by thin section electron microscopy indicated that the cell wall of *Leptotrichia buccalis* showed the typical gram negative cell envelope consisting of a wavy outer membrane, a solid intermediate layer (peptidoglycan), and an inner cytoplasmic membrane (Hofstad and Selvig, 1969). A more recent

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ultrastructural study of *L. buccalis* by Listgarten and Lai (1975) demonstrated that this organism could be distinguished from other gram negative bacilli by scale-like membranous folds superficial to the external lamina of the outer membrane. To date, unique structural features have not been shown in members of the other *Bacteroidaceae*: *Fusobacterium* and *Bacteroides*. Kondo, Sato, and Ozawa (1976) have reported the hemagglutinating activity and the adherence to saliva-coated enamel by *L. buccalis* and suggested that its ability to aggregate and hemagglutinate might be a function of short hair-like surface structures which they observed in the electron microscope. We have been investigating the immunopathologic potential of *F. polymorphum* (*nucleatum*) in the gingival crevice and oral wounds (Hawley and Falkler, 1978) and have reported that the hemagglutinating activity of this organism could be isolated on its cell wall (Falkler and Hawley, 1977). Therefore, we felt that a morphologic examination of *F. polymorphum* would be a valuable adjunct to our current research activities. The detection of surface features, as have been seen with *L. buccalis*, might be relevant to the ability of *F. polymorphum* to colonize the oral cavity and might also facilitate the *in situ* identification of this organism in dental plaque and tissue fluids.

MATERIALS AND METHODS

Growth of Microorganisms: *Fusobacterium polymorphum* (ATCC #10953) was grown under anaerobic conditions at 37°C using the Gas-Pak System (Baltimore Cockeysville, Maryland). A liquid modified tryptone media, pH 7.2,

containing tryptone, 10 g; yeast extract, 10 g; K_2HPO_4 , 1.25 g; $MgSO_4 \cdot 7H_2O$, 1.25 g; glucose, 2 g; and sodium thioglycollate, 5 g in a liter of distilled water was employed.

Electron Microscopy: *Negative stained preparations* of *F. polymorphum* were obtained by using 48 hr broth cultures and employing the techniques of Gregory and Pirie (1973). Micro-drop samples of broth cultures were placed on carbon coated and Formvar (0.25% polyvinyl formal) supported 300 mesh grids and stained with phosphotungstic acid (2.0%, pH 7.4), containing 50 μ g/ml bacitracin as a wetting agent. The air dried grids were then viewed in an aligned and calibrated Siemens Elmiskop electron microscope at an accelerating voltage of 60 kv.

Aliquots of broth cultures (30 ml) were fixed with ruthenium red and osmium tetroxide following the methods of Cagle, Pfister, and Vera (1972). The cells harvested at 10,000 x g for 10 min were treated for 1 hr at room temperature with 1 ml 0.45% ruthenium red in distilled water, 1 ml 0.2 M cacodylate buffer at pH 7.2, and 1 ml distilled water. One ml 0.45% ruthenium red, 1 ml 0.2 M cacodylate buffer, and 1 ml 25% glutaraldehyde in 0.2 M cacodylate buffer were then added, and the mixture was kept at room temperature for an additional hour. The cells were washed twice in a solution containing equal volumes of 0.45% ruthenium red, 0.2 M cacodylate buffer, and distilled water. After the washing step, the cells were fixed again for another hour in 1 ml 0.45% ruthenium red, 1 ml 0.2 M cacodylate buffer, and 1 ml 4% OsO_4 in 0.2 M cacodylate buffer. The cells were washed again in the wash solution containing ruthenium red, and then washed twice more in

0.2 M cacodylate buffer alone. The cell pellet was resuspended in 2% Noble agar at 45°C and pipetted onto a glass microscope slide. After cooling, 1 mm³ blocks were cut and placed into a post-fixation solution of 0.5% uranyl acetate in 0.2 M cacodylate buffer.

The ruthenium red/OsO₄ fixed cells were dehydrated in ethanol and embedded (Spurr, 1969) in Spurr Low Viscosity Embedding Media (Polysciences, Inc., Warrenton, Pa.). Thin sections were collected on Formvar supported 300 mesh grids, stained for 20 min in 50% ethanol saturated with uranyl acetate and then stained for 5 min with lead citrate, pH 12 (Reynolds, 1963). The sections were examined in an aligned and calibrated Siemens Elmiskop 1A electron microscope at an accelerating voltage of 80 kv.

RESULTS

Negatively stained *F. polymorphum* was shown to be straight to slightly curved filamentous bacteria with tapered and rounded ends. The length of the cells varied from 7.0 μ m to 9.5 μ m, and the widths were between 0.5 μ m and 0.6 μ m. Figure 1 shows the complex convoluted surface morphology of the cell. Pili, fimbriae, or organs of motility were not observed in any of the preparations. Frequently, however, the negatively stained cells displayed a polar, circular appendage. The creased or folded surface of these structures gave them the appearance of collapsed membranous sacs. In some preparations, the continuity between the outer membrane of the parent cell and the sac could be visualized (Fig. 2).

The ultra-thin sections of *F.*

polymorphum that had been fixed with ruthenium red and osmium tetroxide are shown in Figure 3. The outer membrane of the gram negative cell envelope showed a wavy pattern with regions of regular periodicity (55 to 60 nm) and amplitude (25 to 30 nm). There were other areas where the wavy pattern of the 7 nm thick outer membrane was absent. The intermediate peptidoglycan layer was more electron dense, had a fine granular character, and had a uniform thickness of 9 to 10 nm. The cytoplasmic membrane (7 nm) was shown in intimate contact with the inner surface of the peptidoglycan layer and did not follow the surface undulations of the outer membrane. A surface layer of coarse electron dense granules measured 9 to 10 nm in thickness and was in close apposition to the external lamina of the outer membrane. The total thickness of the cell envelope, including the surface layer, was 36 nm.

In the same thin sections, a membrane-lined structure was observed (Fig. 4) which corresponded to the collapsed membranous sac seen at the ends of the negatively stained organisms. This appeared in thin sections as an enlarged area of the periplasmic space enclosed by the electron dense surface coat and the outer membrane of the parent cell envelope. The peptidoglycan layer and the cytoplasmic membrane were not involved in the morphology of the structure. The contents of the sac did not resemble the structure of free ribosomes and electron dense inclusions of the cell cytoplasm.

DISCUSSION

The negative stained and thin section preparations of *F.*

polymorphum revealed that this organism has the surface and cell wall characteristics of a gram negative bacterium. Similar findings have been reported by Takagi and Ueyama (1963). Our thin sections of the ruthenium red and osmium tetroxide preparations displayed a surface layer of coarse electron dense granules. Since ruthenium red is believed to react with monosaccharide and polysaccharide groups to form an electron dense reaction product, (Cagle, *et al.*, 1972), the concentration of this histochemical marker against the surface leaflet of the outer membrane suggests the presence of cell surface carbohydrates. This outer coat may play a role in the reported specific tropism of this gram negative anaerobe to the eco-system of the gingival crevice (Loesche, 1969; Socransky and Manganiello, 1971). It has been suggested by Gibbons and van Houte (1971) that surface bacterial coats may be involved in the microbial colonization of the oral cavity through cell to surface and cell to cell attachment mechanisms. The inability to demonstrate surface appendages, such as pili and fimbriae in the negatively stained preparations of *F. polymorphum*, suggests that other surface associated mechanisms, such as this polysaccharide coat, may be responsible for the ability of this organism to agglutinate or adhere to cell surfaces. The retention of *F. polymorphum* in the gingival crevice *via* eucaryotic membrane adherence may be basic to the proposed role of this organism in the immunopathology of periodontal disease (Sabiston and Grigsby, 1972; van Palenstein Helderman, 1975).

The significance of the outer membrane lined periplasmic sac is not totally clear (Figs. 1, 2, and 4).

Recent studies of the gram negative cell envelope indicate that there is a system of hydrolytic enzymes within the periplasmic space (Costerton, Ingram, and Cheng, 1974). These enzymes have an analogy with the lysosomal enzymes of the eucaryotic cell and probably function in an ecologic sense to provide the parent organism with reaction products that are of metabolic significance. Cheng, Ingram, and Costerton (1971) have determined that one of these enzymes, alkaline phosphatase, is electrostatically associated with lipopolysaccharide components of the outer membrane. It has also been shown that lipopolysaccharide-enzyme complexes can be released from growing gram negative bacteria into the surrounding media (Lindsay, Shelagh, Wheeler, Sanderson, and Costerton, 1973). Since attached and free-floating sac-like structures have been observed in broth cultures of *F. polymorphum* by phase-contrast microscopy (Hawley, Falkler, Mongiello, and Zeller, Unpublished), it is possible that the enlarged area of periplasm may eventually pinch off from the organism at some later time in its life cycle or during autolysis of the cell. If this were to occur in the gingival crevice, one can propose a potential damaging biologic effect on periodontal tissue by an export system for lipopolysaccharide and degradative enzymes.

ACKNOWLEDGEMENTS

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MILITARY DISCLAIMER

Commercial materials and equipment are identified in this report to specify the investigative procedures. Such identification does not imply recommendation or endorsement or that the materials and equipment are necessarily the best available for the purpose. Furthermore, the opinions expressed herein are those of the authors and are not to be construed as those of the U.S. Army Medical Department.

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FIGURE 1. Photomicrograph of *F. polymorphum* (ATCC #10953). Cells display the convoluted surface characteristic of gram negative bacteria. The polar periplasmic sac is apparent (arrow) showing surface folds. Print magnification is $\times 67,600$.

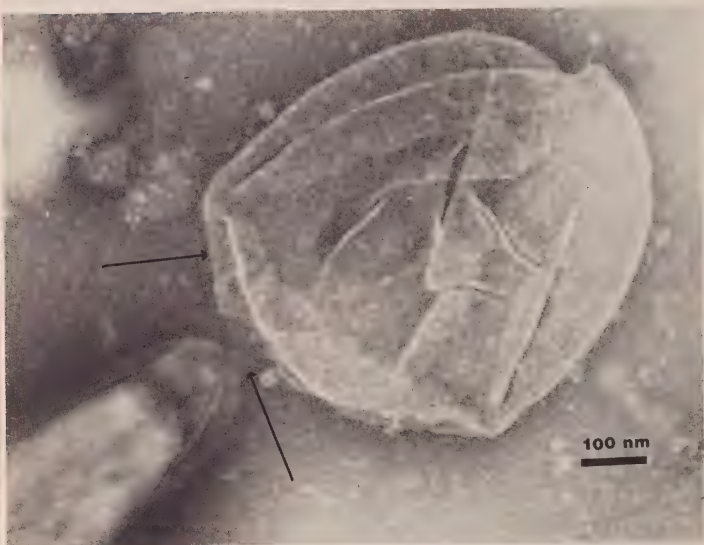
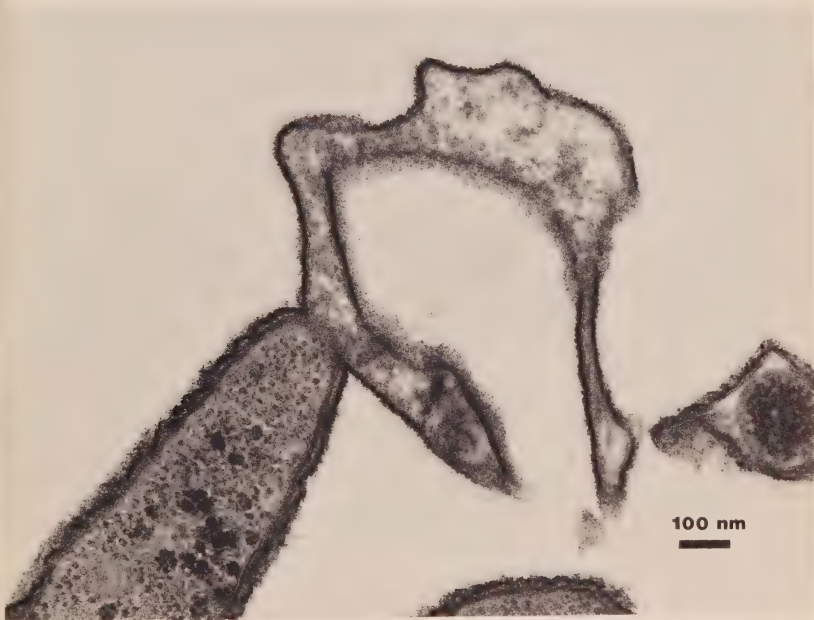


FIGURE 2. *F. polymorphum* (ATCC #10953) negatively stained with phosphotungstic acid showing another polar sac. Arrows designate region of outer membrane continuity between the sac and the parent cell. Print magnification is $\times 128,000$.

FIGURE 3. Photomicrograph of an ultra-thin section of ruthenium red and OsO_4 fixed *F. polymorphum*. The three layers of the gram negative cell envelope are shown. A layer of electron dense granules is apparent external to the outer membrane (arrow). Print magnification is $\times 58,000$.



FIGURE 4. A thin section photomicrograph of ruthenium red and OsO_4 fixed *F. polymorphum*. An outer membrane lined sac is seen at the end of the bacterium. This enlarged area of the periplasmic space is apparently free of cytoplasmic material. Print magnification is $\times 84,000$.



The Office Emergency: Prevention

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The Office Emergency: Prevention

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The American population has developed a higher dental aptitude. Third party payment plans, increased public awareness, more concern for health, and a general increase in longevity have contributed to increasing amounts of dental care sought by the general population. Many of these persons are medically compromised and, therefore, present a greater probability for a life threatening emergency in the dental office.

An emergency in the dental office is a broad term. However, most life threatening emergencies in the dental office occur when the respiratory and/or the cardiovascular systems are compromised through existing pathology in the patient, drugs introduced by the dentist or a combination of both. The American Heart Association estimates that approximately one million persons in the United States experience an acute myocardial infarction each year, while another 650,000 die of ischemic heart disease. About one third of these persons die outside of the hospital within two hours after the onset of symptoms. These people require immediate and proper treatment. Therefore, it is essential

that the dental practitioner be able to recognize the medically compromised patient, quickly and accurately diagnose a life threatening situation, and develop the skill to maintain the life of the patient until supervision of the patient can be turned over to the physician or hospital.¹

The easiest treatment of an office emergency is prevention. The single most important preventive measure that a dentist can perform is to record an accurate and thorough medical history. This history must include a review of systems such as the following:²

1. *Skin*: itching, rash, ulcers, excessive dryness, pigmentary change, changes in hair or nails, hair loss.
2. *Eyes*: vision, inflammation, diplopia, blurring.
3. *Ears, nose, throat*: hearing, earache, epistaxis, sore throat, hoarseness, sinus pain, tinnitus.
4. *Respiratory*: cough, sputum (describe quantity, color, odor, blood) wheezing, infections, exposure to tuberculosis, prior chest x-ray, emphysema,

pneumonia.

5. *Cardiac*: chest pain on exertion, palpitation, dyspnea, orthopnea, swelling of ankles, legs or abdomen. History of rheumatic or scarlet fever, tonsillitis, "heart attack", high blood pressure, murmur.
6. *Gastro-intestinal*: appetite, nausea, vomiting, dysphagia, heartburn, indigestion, food intolerance, abdominal pain, jaundice.
7. *Genito-urinary*: dysuria, nocturia, polyuria, pyuria, hematuria, incontinence, frequency, difficulty starting stream; venereal disease and treatment, kidney infection or kidney stones in past.

For women:

- a. *Menstrual history*: age at onset, last menstrual period and previous menstrual period; dysmenorrhea; other discharge between periods.
- b. *Menopause*: age of occurrence, hot flushes.
- c. *Obstetrical history*: pregnancies, miscarriages, deliveries, still births, living children.
8. *Endocrine*: diabetes, hepatitis, hyper/hypo thyroid or parathyroid, longterm steroid therapy.
9. *Extremities*:
 - a. *Vascular*: varicose veins, phlebitis, CVA.
 - b. *Joints*: pain, stiffness, swelling of joints, pain in the back or neck, arthritis.
 - c. *Muscles*: weakness, pain, tenderness, cramps.

10. *Nervous System*: syncope, convulsions, headache, vertigo, tremor, weakness, paralysis, paresthesias, anesthetics, epilepsy.

11. *Hematopoietic*: bleeding tendency, excessive bruising, anemia and treatment, transfusions in past (date, reaction), known exposure to radiation or toxic agents.

12. *Psychiatric*: "nervousness", irritability, depression, history of previous "nervous breakdown".

The history must include all known hospitalizations and serious illnesses as well as any known drug allergies with particular questioning as to drugs commonly used in dentistry such as penicillin, local anesthetics and aspirin. The dentist must know all medications that the patient is taking whether prescribed or over the counter in order to prevent any possible adverse drug interactions. Other significant information includes a family and social history. In the family history, the dentist must note any familiar tendencies or inherited diseases such as hemophilia, diabetes, malignant hyperthermia, cancer, adverse reaction to any drug or anesthetic and any other disease with hereditary tendencies.²

Social history should include occupation, marital status, habits and a general evaluation of a patient's homelife and activities. Important habits to know are smoking, use of alcohol and drug abuse. The degree and duration of the habit must be evaluated since heavy smoking, alcoholism and drug abuse of any kind for a prolonged period of time can significantly compromise the medical status of the patient.²

This essential information cannot be compiled with a single fill-in-the-blank medical form because patients tend to take such a history too lightly, while the dentist cannot possibly obtain the vital information about the patient's existing medical status. The dentist must confront the patient face to face, establish a personal rapport, ask the necessary questions, record the necessary information; and if needed, explain the necessity for asking these questions to the patient. Ten or fifteen minutes invested in seeking such a history could prevent a needless death in the dental office.²

Another preventive measure that a dentist can simply perform in the office is the recording of basic vital signs such as blood pressure, pulse, and temperature. While the temperature need only be taken on patients with suspected systemic infection, it is mandatory to take base line blood pressure and pulse readings on every patient as "undiagnosed hypertension is rampant, affecting approximately five per cent of the adult population". (Robbins, 1971) The possible combination of an uncontrolled hypertensive patient, pain, and the increased stress felt prior to a dental procedure could precipitate a life threatening situation.³

The final and most important stage in the prevention of a medical emergency is the dentist's evaluation of the patient's overall medical status. Any medical condition that the dentist suspects could be potentially serious should be documented and dental treatment should be postponed, if at all possible, until a medical consultation from the patient's physician can be obtained. Any elective procedure should definitely be postponed until results of a medical consultation are received

and the physician and dentist have agreed that the treatment plan will not jeopardize the patient's health status. If the patient is so compromised medically that any dental treatment rendered in the office would be life threatening, the patient should be referred for dental treatment in a hospital where there is a controlled environment and all necessary life support systems are available.

A thorough medical history, proper interpretation, and the recording of vital signs significantly reduce the probability of a medical emergency in the dental office. However, unexpected medical emergencies arise despite all of these precautions. These emergencies can range from syncope to cardiac arrest. The dental practitioner must be able to quickly and accurately render proper treatment. It is absolutely mandatory that every dental practitioner and his staff be competent in basic life support, i.e., Cardiopulmonary Resuscitation (CPR) and an adequate emergency kit must be maintained. This kit should include a passive and positive pressure oxygen system, materials for airway maintenance, and the essential drugs necessary for basic life support as recommended by the American Heart Association.

Any practitioner who chooses to use any type of sedation be it N₂O-O₂ or IM or I.v. sedation, must be fully competent at not only basic life support but be skilled in advanced life support as well. This includes establishing and maintaining an I.V. lifeline, knowledge of the pharmacology and administration of cardiac supportive drugs, airway maintenance including endotracheal intubation and cardiac

monitoring including interpretation of basic cardiac life threatening arrhythmias.¹

A thorough knowledge of a patient's health status allows a practitioner to render the best dental care at the least risk to the patient. The health history, recording of the vital signs, and the proper interpretation of the data obtained help the practitioner: screen out potential serious medical complications reducing the probability of a life threatening situation; evaluate the need for medical consultations; select those patients whose medical condition warrant admission to a hospital for dental treatment; and prevent infection of the dentist, his staff and his other patients by serious communicable diseases.

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Note to Editor

**A Synopsis of Evolutionary Events
Leading to the Masticatory Apparatus
in Modern Man**

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Note to Editor

A Synopsis of Evolutionary Events Leading to the Masticatory Apparatus in Modern Man

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So careful of the type she seems,
So careless of the single life.

Tennyson

INTRODUCTION

As our vertebrate ancestors moved from an aquatic to a terrestrial domain and, subsequently, to an arboreal existence before returning to a terrestrial life style, adaptation to environment required extreme changes in the mechanisms for sensing, feeding and locomotion. Adaptive changes in the masticatory apparatus have preceeded or accompanied all of these major vertebrate adaptations to environment.

Table I summarizes the sequence of vertebrate evolution. Man, an improbable product of 600 million years of evolution, is a relative newcomer to the scene, having differentiated only within the past 1 million years. However, our structural and functional heritage can be traced to those earliest vertebrate forms which emerged during the Paleozoic Era. Because fossilized teeth, jaws and skulls offer well documented paleontologic evidence, it is possible to reconstruct a rather comprehensive sequential account of the evolution of the masticatory apparatus.

Adapted from the postgraduate lecture series on Growth and Development, Advanced Specialty Education, Dental School, University of Maryland, 1977.

TABLE I
EVENTS IN HUMAN EVOLUTION APPROXIMATELY DATED
MILLIONS OF YEARS

<i>Stratigraphical Phases of the Earth's History</i>		<i>Extent</i>	<i>Beginning Before Present (B.P.)</i>	<i>Event</i>
Quaternary	Pleistocene	3	3	Rise of man
	Pliocene	10	13	Rise of hominids
	Miocene	12	25	
	Oligocene	11	36	Rise of monkeys
	Eocene	22	58	and apes
Tertiary	Paleocene	5	63	Rise of placental mammals including primates
	Cretaceous	72	135	First placental mammals; extinction of ruling reptiles
	Jurassic	48	181	First mammals, the pantotheres
	Triassic	49	230	
Mesozoic	Permian	50	280	
	Carboniferous	65	345	First reptiles
	Devonian	60	405	First amphibians
	Silurian	20	425	Fish
	Ordovician	75	500	
Paleozoic	Cambrian	?100	?600	First fossilized animals

After Campbell

AGNATHA

Primitive jawless fish (Agnatha) originated during the Silurian Period. These fish-like creatures represent the earliest and simplest forms of vertebrate life. Sedentary creatures, capable of only limited swimming motion because of the lack of dorsal and pectoral fins, early Agnatha subsisted on food ingested directly into the gut through a suctorial mouth. Later forms of Agnatha developed cartilaginous branchial arch skeletons. Dentin appeared in the dermal

scales of Agnatha and represents one of the earliest known calcified structures in vertebrate evolution.

Agnatha, except the most primitive forms, were filter-feeders; material sucked into the mouth from the aquatic environment was filtered through the gills which served as sieves or rakers, combing out and retaining food particles and passing them into the gut. Movement of the gills was controlled by muscles of the body segment. As posterior modifications increased

the mobility of Agnatha, the demands for more aggressive food gathering increased. Gradually, the suctorial mouth became infolded, merging into the first branchial arch to produce a grasping jaw; the apex of the branchial arch formed the jaw joint, the cartilage of the upper arm of the arch becoming the skeletal base of the upper jaw, the lower arm of the arch becoming the lower jaw skeleton; the muscles controlling gill movement became the adductor muscles of the jaws; and the skin overlying the invaginated suctorial mouth specialized to form primitive teeth or barbed hooks. The joint of the primitive jaw was of the simplest type, a fibrous connector (synarthrosis).

GNATHOSTOMES

The transition from Agnatha to Chondrichthyes (fish with cartilaginous skeletons) is significant, from the dental viewpoint, in several respects: it established the basic identifying characteristics of the phylum Chordata, a notochord and branchial arches; it introduced the basic processes of formation of cartilaginous internal skeletal structures as protection for the anterior portion of the neural cord and as support for the branchial arches; it introduced the formation of dermal scales or denticles which, as the mouth invaginated, produced enamel from their epithelial surfaces and dentin from the underlying mesenchyme, a primitive method of tooth formation utilizing an *eversion* of the enamel organ.

As jaw-bearing fish (Gnathostomes) evolved through the stages of cartilaginous skeletons to fish with true bony skeletons (Osteichthyes) during the Devonian Period, pectoral and pelvic fins increased their locomotion with

resulting changes in feeding habits. Active predation required more elaborate protection of the neural tube at its anterior extremity (the brain): the cartilaginous brain covering became progressively encased in dermal (membranous) bone which, on the underside of the brain case, also formed the primary palate. The cartilaginous bars of the first branchial arch which served as upper and lower jaws, articulating at the apex of the arch, required reinforcement: the simple synarthrosis was replaced by a sturdier connective tissue joint (schizarthrosis) uniting the cartilage bars; the second branchial arch cartilages moved forward to provide hyostylic bracing for the previously unsupported jaws. The cartilaginous bars of the primitive jaws required reinforcement: dermal bone began to envelope or sheathe the anterior portions of these bars. Teeth, originally situated upon the soft tissue orifices of the mouth, became more actively involved in seizing and grasping; they migrated to the marginal areas of the jaws and developed complexly infolded bases (Labryinthodont) which, although lacking roots, rested in grooves of the posteriorly enlarging dermal bone. It was at this early evolutionary stage that teeth and scales became completely differentiated and vertebrates acquired a horizontal overlap of upper over lower teeth. Also, at this stage of evolution, the muscles controlling simple, hingelike jaw movements were essentially the muscles of the first gill arch.

The rise of true bony fish during the Devonian Period was accompanied by increasingly forceful predatory feeding habits which impaired the stability of the hyostylic jaws. In response to increased functional demands the hyomandibular-

supported jaw joint migrated superiorly, bracing itself against the palatoquadrate bone of the cranial base, thus providing an autostylic jaw. Simultaneously, the connective tissue joint adapted to the demands of increased force and assumed the structure characteristic of a eudiarthroidal joint: the articular surfaces of hyaline cartilage became overlaid by fibrocartilage; the joint cavity became defined within a two part synovial membrane, and the joint capsule became well differentiated with a strong bracing ligament. DuBrul identifies these characteristics as the pattern upon which the temporomandibular joint of mammals would ultimately be designed.

During this transitional period in evolution of the jaw joint the teeth underwent surprisingly little change, remaining as barbed hooks which provided only a grasping and holding function. Tooth and joint structure of the bony fish persisted as the feathery-winged Crossopterygian fishes made the transition from aquatic to terrestrial habitat; however, extensive changes occurred in other bodily structures as the transition was made from active swimming predator to tetrapod and from gill breathing to a lung breathing mechanism. Also, of necessity, the early, slow moving terrestrial forms were reduced to an insectivorous diet.

REPTILES

As early Permian reptilian forms developed and evolved, a lengthening of the legs increased the locomotion of the vertebrate. Feedings habits changed from insectivorous to carnivorous. Food gathering

technics required speedy capture of prey with a snapping jaw closure and forceful holding action, made more difficult by the absence of an aquatic environment. Rather extensive adaptations of the stomatognathic system were required.

Adaptation of Teeth in Reptiles

Early reptilian teeth lost their barb-like shape, becoming peg-like in form (Cynodont) and partially embedded in bone (protothecodont). Later reptiles evolved a heterodont dentition with differentiation of teeth into incisors, canines, premolars and molars. Teeth became rooted in bony sockets (thecodont); anterior teeth developed single roots, posterior teeth became double rooted; a deciduous and a permanent series of teeth emerged.

In the Triassic stages of reptilian evolution, peg-like tooth forms became modified. The cheek teeth became laterally compressed and incipiently triconodont with a high central cusp and a small anterior and posterior cusp. Horizontal and vertical overlap of the teeth developed. Occlusion was of an advanced therapsid or theriodont type, i.e., upper teeth alternately lying between lower providing alternately interdigitating frictional wedges with a fierce, wild beast-like appearance.

Adaptation of Jaw Joints in Reptiles

The autostylic jaw joint evolved by true bony fishes persisted unchanged throughout most of the period of reptilian evolution, with the ossified proximal end of the mandibular cartilage of the first branchial arch (Meckel's cartilage) articulating against the palatoquadrate bone of the skull (the articular-quadrate joint or primitive jaw joint). This

joint functioned purely as a locked hinge.

Adaptation of Muscles and Their Bony Insertions

By far the most significant structural changes in the stomatognathic system during reptilian evolution relate to muscles of the jaws and to their bony insertions.

Speed and power of jaw closure were essential to the survival of carnivorous, terrestrial reptiles. The cartilaginous skeletal bars of earlier vertebrate jaws had become sheathed anteriorly by dermal (membranous) bone, providing attachment for the teeth. Residual musculature of the first branchial arch, attached in the region of the ossified articular end of Meckel's cartilage, had served as the mandibular adductors. As dermal bone began to spread lateral to Meckel's cartilage in the area of the lower jaw distal to the teeth, it provided attachment for a mass of muscle, the temporal mass, originating from the inner aspect of the lateral wall of the skull. The temporal mass provided adductor power to the mandibles of bony fishes and, later, became the primary mandibular adductor of the reptile. Muscle length (and power) was limited by the elevated position of the mandible, wedged almost as a horizontal rod within the confines of the maxillary arch.

Vertical growth of the reptilian skull permitted the formation of a secondary palate and placed the pterygoid fossa of the sphenoid bone in juxtaposition to the temporal mass which, in turn, proliferated into the fossa, enhancing adductor speed and power. Further, in response to increased activity of the temporal mass, the lateral aspect of the bony cranial compartment

fenestrated, permitting the temporal mass to proliferate and attach to the outer surface of the skull. The ledge of bone which remained inferior to the temporal fenestration marked the beginning of the zygomatic arch in vertebrate evolution.

As dentary bone expanded lateral to Meckel's cartilage, it also expanded superiorly producing a coronoid-like process on the mandible for attachment of the temporal mass. Superior enlargement of the dentary also resulted in the mandible's dropping downward, away from the maxilla. The temporal mass within the pterygoid fossa migrated inferiorly, attaching to the medial surface of the lowered mandible to form the medial pterygoid muscle. In later reptiles, the mandible descended further below the maxilla; the temporal mass promptly migrated down the lateral surface of exposed dentary to form the masseter muscle. These three primitive muscles, the temporal, medial pterygoid and masseter, arising from a common source in the temporal mass, laid the groundwork for future mammalian masticatory muscles. The temporal muscle, favored by its length and attachment to the coronoid-like process of the expanding dentary, was primarily an elevator muscle designed for speedy jaw closure; the masseter and medial pterygoid, short and more anteriorly positioned, functioned as power elevators and holding muscles for the mandible. As yet, the expanding dentary bone had not made contact with the base of the skull and the lateral pterygoid muscle had not appeared. No lateral movement of the mandible was necessary or possible; the "locked-slot" nature of the jaw joint permitted only rotational movement although the unfused mandibular

symphysis did permit some slight lateral spread of the mandibular bodies, enhancing gripping action of the posterior teeth.

MAMMALS

From the viewpoint of those concerned with the stomatognathic system, the transition from reptilian to mammalian vertebrate forms introduced highly significant structural changes in the jaws and teeth resulting primarily from altered feeding habits associated with remodeling of the neck and skull. The principal structural changes of dental interest, again, are reflected in the jaw joints, the musculature and the teeth.

Modifications of the Jaw Joint and Musculature in Early Mammals

The articular-quadrate joint of the reptilian jaw persisted into the early stages of mammalian evolution. As the enlarging dentary bone of the mandible, which had enveloped Meckel's cartilage in its anterior portion, began to expand laterally and superior to the posterior portion of the primitive cartilage bar, it approached the inferior-lateral aspect of the cranial squamous temporal bone. From this position lateral to the skull, the dentary began a medial direction of growth, approaching and contacting the squamous temporal to establish a "new" jaw joint. Intervening fibres of the temporal muscle mass were trapped between the medial aspect of the growing condyle and the squamous temporal; the compressed muscle fibres became the medial half of the articular disc of the new joint; the non-compressed fibres anterior to the point of entrapment became the lateral pterygoid muscle and, as skull growth progressed, these

fibres assumed an oblique latero-medial direction. By proliferation, this "new" muscle gained attachment to the neck of the new condyle. Also, rotation of the pterygoid fossa to a posteriorly facing position exposed the lateral lamina as a site for muscle insertion almost directly medial to the newly formed condyle; thus the lateral pterygoid muscle originated as a mandibular adductor, stabilizing the condyles against lateral displacement in their grooved fossae. During later evolutionary changes, persisting into the human masticatory system, the lateral pterygoids maintained this primitive adductor function in a modified form.

With the development of a new eudiarthroidal joint between dentary and squamous temporal bones, the more primitive jaw joint became unneeded. The remnants of Meckel's cartilage assumed new functions: that portion remaining anterior to the articular elements formed the sphenomandibular ligament; the articular components migrated medially to become the malleolar-incus ossicles of the middle ear, carrying with them one of the muscles of the primitive jaw. This muscle became the tensor tympani muscle and carried with it its innervation by the 5th cranial nerve. Reichert's cartilage of the second branchial arch, formerly providing hyostylic bracing for the jaw joint, also persisted and assumed new functions, forming the stapes, the stapedius muscle, the styloid process, the stylohyoid ligament and the lesser horn of the hyoid bone. The musculature of the second branchial arch became the muscles of facial expression and carried with them the original innervation by the 7th cranial nerve.

With the disappearance of the "old" reptilian jaw joint, the "new" temporomandibular joint became a typical eudiarthroidal structure.

Modifications of the Teeth in Early Mammals

With the advent of the "new" temporomandibular joint, early mammals underwent a change in morphology and function of the reptilian tricone shaped posterior tooth. The laterally compressed posterior tooth of the reptile consisted of one or more secondary cusps situated upon the mesial and distal inclines of a high central cusp. Upper teeth lay buccal to the lower. In late reptiles, the secondary cusps enlarged, and, on the upper teeth, migrated lingually; on the lower teeth the enlarged secondary cusps migrated buccally. The resulting tooth presented a trigon or tricuspid form, permitting interdigitation of opposed teeth. This trigon or tritubercular occlusal morphology resulted in a tribosphenic (shearing wedge) occlusion and formed the basic pattern for most subsequent mammalian occlusion. Later in mammalian evolution, the development of a talon or ledge at the back of the tricuspid molars enlarged anteroposterior width of the teeth and gave rise to additional cusps. In 1888, H. F. Osborn published a theory of cusp evolution, the Tritubercular Theory, based upon transformations of the trigon tooth form. This theory applied only to the posterior teeth of later mammalian orders. Today, the history of mammalian tooth development and the pertinent nomenclature remain highly controversial.

Modifications Accompanying Adaptive Radiation of Mammals

As basic mammalian patterns

emerged, environmental factors again exerted significant influences upon the stomatognathic system. Elective and imposed dietary sources rapidly differentiated most mammals into two extremes of feeding behaviour, carnivorous and herbivorous. A few developed omnivorous feeding habits.

Late mammal-like reptiles had been carnivorous creatures with suitably adapted masticatory mechanisms: a eudiarthroidal jaw joint, the condyle of the mandible "locked" into a slot-like fossa of squamous temporal bone, permitting a wide gape with only a hinge movement of the mandible; a large coronoid process providing attachment for a large temporal muscle; medial pterygoid and masseter muscles with vectors of force between origins and insertions providing strong holding force; a lateral pterygoid muscle exerting a medial pull upon the condylar processes; posterior teeth which were in process of transformation to intercusping occlusion; and anterior teeth well-differentiated into centrals, laterals and canines. Mammalian carnivores required little adaptive change. Functionally, the dentition was designed for predation, rending and tearing: mandibular canines prominent above the occlusal plane, controlled by the adductor muscles and occluding in a diastema between maxillary lateral and canine; maxillary canines prominent, extending below the occlusal plane and controlled by the cervical musculature; central and lateral incisors large and pointed; posterior teeth, short rooted and multibladed but positioned forward of the relatively small masseter-ptyergoid complex, were unembarrassed by a cheek.

Other mammals experienced extensive modification of the entire masticatory apparatus in progressing from a carnivorous to an herbivorous diet. Incisor teeth diminished in size or disappeared; canine teeth became reduced to the level of the occlusal plane; posterior teeth developed broad, convoluted occlusal tables and long supporting roots. The temporal muscle, unneeded for speedy jaw closure, diminished in size while the masseter-ptyergoid complex enlarged and migrated to a more anterior attachment, the masseter attachment to the zygomatic process advancing anteriorly to such a degree that the anterior fibres were in a position to function as protractors as well as elevators of the mandible. The lateral pterygoid muscle began to exert an influence on mandibular movements. In response to functional demands, the locked condyle-fossa relation remodelled to permit limited anterior gliding movements; the medial wall of the fossa modified progressively to permit extensive lateral movement of the condyles. The diminished size of the coronoid process and temporal muscle and a posterior migration of the cranial attachment of the temporal muscle enabled this muscle to serve as a retractor of the mandible in concert with the pterygoid-masseter complex. The primary function of the lateral pterygoid, however, consisted of producing the alternative lateral shift of the mandible necessary for a milling type of masticatory mechanism.

Since all mammalian masticatory muscles evolved from the primitive temporal mass and since this mass was designed for speedy capture as well as for powerful grasping of prey, it seems logical that all

muscles derived from the temporal mass should be well endowed with stretch receptors. It is interesting to speculate that the lateral pterygoid muscle, originally well supplied with stretch receptors, gradually lost most of these receptors when, because of its altered direction of force, it became an antagonist of the mandibular elevators. Now, it became necessary for the lateral pterygoids to reciprocally relax as the elevators forcefully and suddenly contracted; the myotactic reflex diminished and the golgi tendon apparatus became the paramount neural mechanism of the lateral pterygoid, assuring the integrity of the muscle.

THE RISE OF MAN

Although the sequence of ancestry of modern man is, in some measure, hypothetical, it is currently accepted that man diversified from the basic primitive stock of arboreal mammals and, after returning to a terrestrial existence, acquired an upright posture. Environmental factors related to available dietary substances played a significant role in modifying the masticatory mechanism. Anthropology suggests that after descending from a frugivorous-insectivorous arboreal existence, hominids duplicated the two extremes of masticatory adaptation (herbivorous and carnivorous) experienced by earlier mammals. Dietary factors, however, exerted only a secondary effect; modifications imposed upon body structures by the newly acquired bipedal locomotion produced the most profound masticatory changes. The jaws were freed of aggressive, prehensile activity and the cranium remodelled to provide a scanning balance of the head upon the vertebral column. The snout-type face began to gradually

retrude, bringing the center of gravity of the head nearly in line with the occipital condyles. Simultaneously, vaulting of the dorsum of the skull began. In effect, the previously elongated skull of reptiles and mammals became bent downward and inward around the sella turcica, forcing the pharyngeal structures downward and backward as the masticatory complex retruded. As the occipital region of the skull bent downward and forward, the dorsum enlarged superiorly. With the advent of modern man in the evolutionary picture, the facial profile became essentially vertical. The posteriorly descending cranium had developed an anteriorly inclined mastoid process which, in the absence of ventral muscles to support the skull, provided attachment for the sternomastoid muscle in an effort, only partially successful, to stabilize the skull upon the vertebral axis. At this point, pure rotation of the mandibular condyles about a fixed transverse axis, a characteristic of earlier primates, became altered. In modern man, pure rotary opening of the mandible became limited as the posterior border of the vertical ramus impinged the soft tissue distal to it against the mastoid process. Further opening required an abrupt involuntary anterior translation of the condyles by the lateral pterygoid muscles as is graphically depicted in Posselt's tracings of the envelope of motion of the mandible. Further opening of the mandible became a function of the digastric muscles, producing the characteristic posterior progression of the mandible in maximal opening. In effect, interruption of pure rotary opening of the mandible created two transverse axes of opening: the axis for initial opening passing horizontally through the

resting condyles; the axis for posterior translation passing through the vertical ramus on a level approximating that of the occlusal plane of the dentition. The site of the axis for secondary vertical rotation served as an ideal point of entry of the mandibular nerve into the lower jaw since this area subjected the nerve to minimal displacement, even during extensive jaw movements.

The mechanisms for mandibular protraction became increasingly molded as mammalian herbivores developed. With modifications, these mechanisms persisted into modern man. Accompanying a deepening of the vertical height of the jaws and accentuated by the retrusion of the masticatory complex, in man, the lateral pterygoid muscles assumed an obliquely lateral and posterior direction between origins and insertions. The new function of the lateral pterygoids, protraction of the mandible, did not eliminate the primitive function of the muscles; they still retained an adductor function, drawing the condylar components of the mandible toward each other. This latter action is more pronounced during forceful grinding movements and in forced protraction of the mandible, when the direction of force of the lateral pterygoids approaches a right angle to the sagittal plane. During such forceful protraction, a measurable narrowing of .5mm occurs across the width of the mandible in the third molar area as a result of bending of the symphysis. DuBrul and Sicher have proposed that the inferior border of the mandible, particularly the area of the symphysis, everted as a buttress against this bending action; at the same time eversion of the chin and

inferior border of the mandible provided greater freedom for the cervical viscera. The mandibles of previous primates were provided with lingually projecting bony buttresses, the simian shelf or mandibular torus. An in-depth review of this aspect of evolution is fundamental to an understanding by the complete denture prosthetist of the basal seat area in advanced alveolar resorption of the mandibular ridge.

Vaulting of the cranial dorsum in mammals was accentuated in man by the progressive growth of the brain and the resulting structural alterations of the skull produced adaptive changes in the masticatory system. A far greater impact upon the continuing evolution of man was exerted by the intellectual faculties derived from an expanded cerebral cortex.

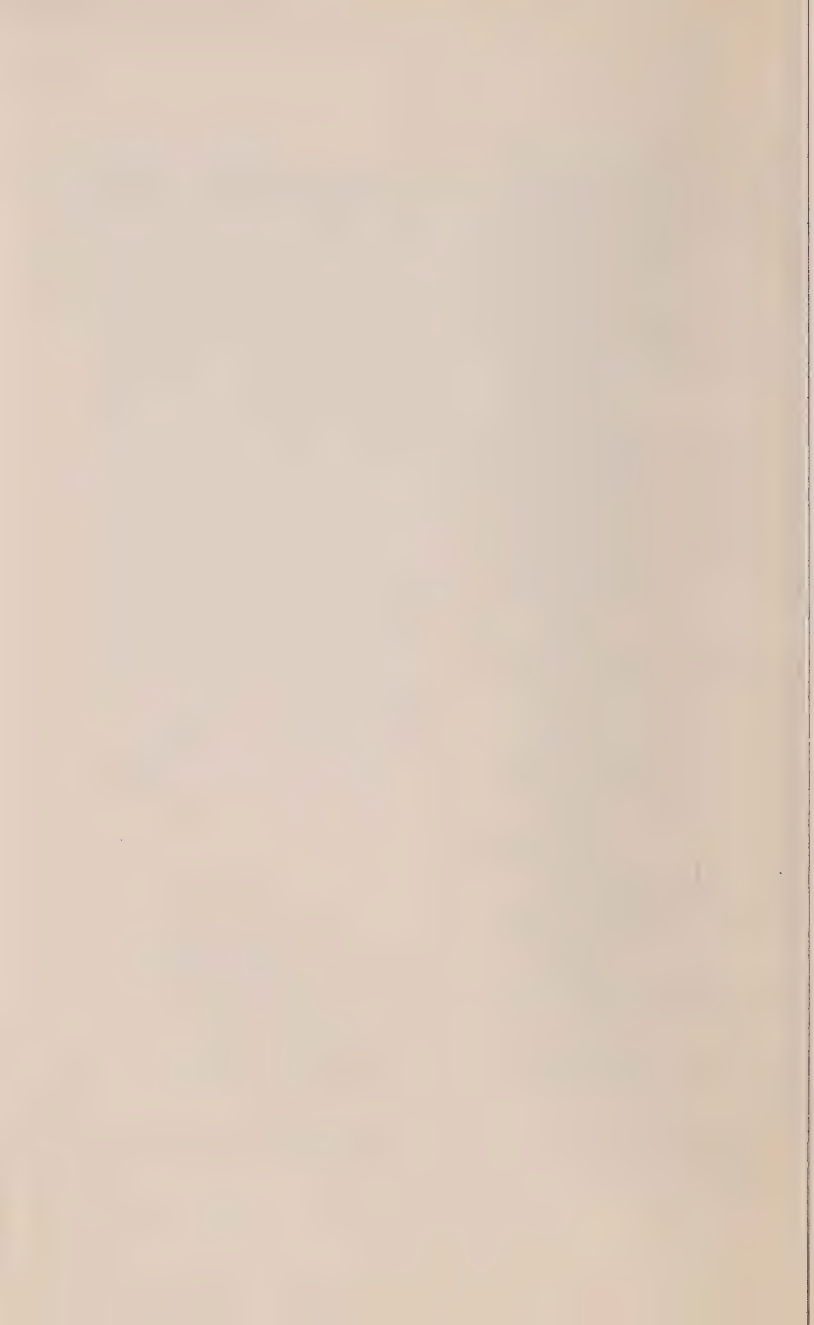
CULTURAL EFFECTS UPON THE MASTICATORY APPARATUS

Early man, of necessity, became a societal animal. Manual and digital skills, the development of speech and the organization of social institutions represent outcomes of

organized, conceptual thinking collectively identified as "culture". Today, the evolution of man continues as a response to environmental factors. However, the significance of environmental change has been overshadowed by the influences of cultural development. These uniquely human cultural adaptations have tended to isolate man from his external environment and have served as a means of preserving his society. Masticatory mechanisms, originally developed as adaptations to the external environment, are no longer operative or are operative in only a vastly altered manner. Dental decay, periodontal disease, malalignment of teeth and osseous structures, the so-called temporomandibular joint disorders and cranio-facial anomalies may be viewed from a different perspective if we consider them as products of a culturally imposed environment. Genetics, of course, has played an operative role throughout the evolutionary process. Genetics continues to influence the evolutionary process in man; however, its effects have been intensified by cultural processes.

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Dr. Robert K. Nauman, Ph.D., Associate Professor, Microbiology uses one of three transmission electron microscopes available in the Dental School for research.

Photo:

Frank F. Elliott, Jr.



This issue of the *Journal* brings with it not only a completely new appearance and editorial structure, but a transfer of the publication responsibilities from Dr. D. Vincent Provenza to Dr. James F. Craig.

Dr. Provenza, Professor and Chairman of the Department of Anatomy at the Baltimore College of Dental Surgery, Dental School, University of Maryland at Baltimore, served as Editor of the *Journal* since 1965. He relinquishes this responsibility to accommodate the pressures of textbook revisions.

Under his editorship the *Journal* has received national and international recognition.

The *Journal* and its staff have been fortunate to have had the leadership and guidance provided by Dr. Provenza over the past fifteen years. Sincere thanks and best wishes for continued success are extended.

IgA Concentrations in Parotid Saliva of Alcoholics and Head and Neck Cancer Patients

Neil D. Hollyfield
Frederick G. Smith
William A. Falkler, Jr.
Paul D. Thut

ABSTRACT

IgA and IgG levels were measured in parotid saliva samples from alcoholics, head and neck cancer patients and a control population. IgG was observed in only one parotid saliva sample. A higher level of IgA was observed in young alcoholic patients than in age matched controls. The concentration of IgA in saliva of older alcoholics was less than that in controls while IgA levels in head and neck cancer patients who had undergone treatment were not different from that in the older control population. The duration of alcoholism, degree of cigarette consumption and time of abstinence from alcohol were associated with changes in the salivary IgA levels of patients.

INTRODUCTION

Saliva contains a secretory immunoglobulin molecule called secretory IgA (sIgA). The bathing of mucosal surfaces by this antibody molecule is thought to be an important mechanism in host defense against bacteria and viruses (Adinolfi, Glynn, Lindsay, and Milne, 1966; Ogra, Karson, Righthand, and MacGillivray, 1968; Tomasi and Bienenstock, 1968; McClelland, Samson, Parkin and Shearman, 1972 and Waldman, Jurgensen, Olsen, Ganguly, and Johnson, 1973). The oral health of alcoholics in general is very poor and a relationship between alcoholism and head and neck cancer has been established (Lowry, 1975). The levels of sIgA in saliva of patients may be valuable in establishing the role of IgA in oral health and disease states. In this research the concentrations of IgA and IgG in parotid saliva were measured in alcoholics (upon admission and release from an alcoholism detoxification center), head and neck cancer patients and a control population of oral health care professionals.

MATERIALS AND METHODS

Patient Selection. Saliva samples were obtained from 24 diagnosed alcoholics upon their admission to a short term alcohol detoxification center (Tuerk House, Baltimore, Md.). The patients' ages ranged from 23 to 64 years, with a mean of 36.2 years. IgA data which have been presented were derived only from those patients which were free of infection and the salivary samples were obtained free from any contamination. Salivary samples were again collected 10 days after admission. Patients which left the center for any time or any reason were not used in comparison between day 1 and day 10 samples. Six male and three female head and neck cancer patients, ages 36-73 years (average age 57.5 years) were also included in this study. All patients had squamous cell carcinoma of the larynx and had received some treatment for their cancer prior to saliva collection. Two patients were treated with irradiation whereas the remaining underwent surgical treatment. The control population consisted of 20 male oral health care professionals, age range 21-41 (average age 27.0 years). This group had good oral health and neither smoked nor drank excessively.

Collection of Saliva. Paraffin stimulated parotid saliva samples were collected from the opening of Stenson's duct, using previously sterilized modified Curby cups (Figure 1). The saliva samples were collected into sterile tubes two hours after the noon meal in the alcoholic and control populations and approximately two hours after the morning meal with the cancer patients. Immediately after collection, the saliva samples were frozen and stored at -20°C .



Figure 1: The modified Curby cup in position over Stenson's duct. A marks the vacuum port, B and C the saliva evacuation ports.

Quantitative measurement of IgA and IgG in saliva samples. IgA and IgG immunoglobulin levels were quantitated by the use of Behring low level IgA and IgG radial immunodiffusion plates, monospecific for alpha heavy chain and gamma heavy chain, respectively. After placing the standards and saliva specimens into the wells on the plate, the plates were incubated at room temperature for 64 hr prior to reading. The diameters of the precipitin rings of the standards and saliva samples were measured with a vernier gauged caliper and recorded. The square of the diameters of the precipitin rings of the standards were plotted against their concentrations and this plot was used to determine the concentration of the saliva samples. The concentration of IgA was recorded in International Units (I.U.)/ml, one I.U. equal to 1.66 mg. ± 1 S.E.M.

RESULTS

With the exception of the saliva sample from one individual from the student population, no IgG, an indication of serum contamination, could be quantitated in any of the parotid saliva samples. The sample containing IgG was not used in this study. Both the control and alcoholic populations were divided by age into two groups. One group had ages less than 30 years while the other had ages 30 and older. As can be observed in Figure 2, the mean IgA concentration of the young control population was 1.52 ± 0.23 international units (I.U.)/ml of parotid saliva. The alcoholics under age 30 displayed an IgA level, upon admission, that was 3.5 times higher than the controls ($P < 0.025$). The IgA concentrations in the saliva of treated head and neck cancer patients were not significantly different from those of the controls.

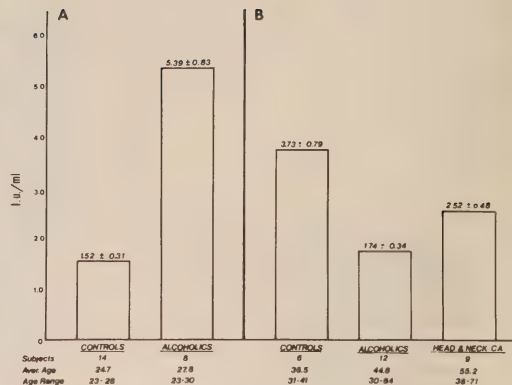


Figure 2: Parotid salivary IgA concentrations of controls, alcoholics, and head and neck cancer patients. Panel A represents individuals with ages less than 30. Panel B represents patients with ages of 30 years or greater.

After 10 days of abstinence there was a significant ($P < 0.001$) reduction in the salivary concentrations of IgA in the younger alcoholic group (Figure 3). The observed concentrations were approximately one-half of those observed upon admission. The mean IgA concentrations of alcoholics after 10 days of abstinence were no longer significantly elevated. There was no significant reduction of secretory IgA levels in the older alcoholics following 10 days of abstinence.

The effect of cigarette consumption of the alcoholics IgA level was studied. The alcoholics who smoked less than 2 packs per day showed significantly higher levels of IgA (2.72 ± 0.75 I.U./ml)

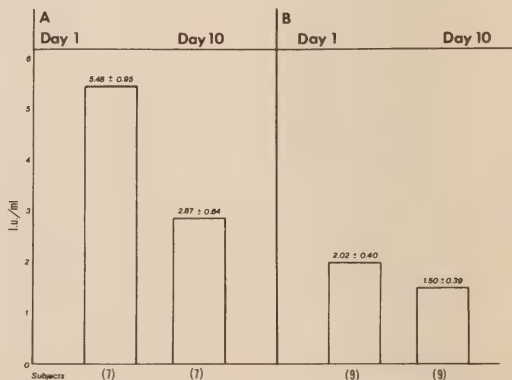


Figure 3: Parotid salivary IgA concentrations in alcoholics on admission and after 10 days of alcohol abstinence. Panel A represents individuals with ages less than 30 years. Panel B represents patients with ages of 30 years or greater.

than those who smoked 2 or more packs per day (1.00 ± 0.29 I.U./ml).

The effect of duration of alcoholism on IgA levels was observed. Those individuals who drank excessively for more than ten years showed significantly ($P < 0.05$) lower IgA levels than those drinking ten years or less (Figure 4). This difference was

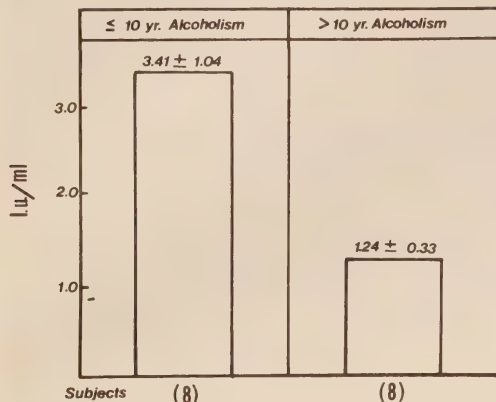


Figure 4: Parotid salivary IgA levels and duration of alcoholism.

even more pronounced in the alcoholics less than 45 years of age (Figure 5). Significance of this difference could not be demonstrated due to the small

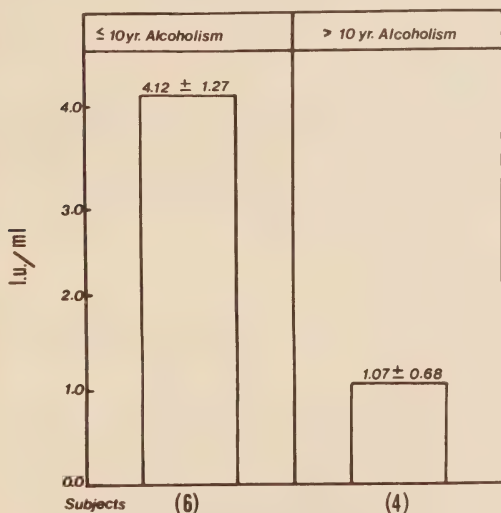


Figure 5: Parotid salivary IgA levels and duration of alcoholism in alcoholics less than 45 years of age.

size of the group which was ≤ 45 years of age and had been alcoholic for > 10 years.

DISCUSSION

A definite correlation has been made between the excessive use of alcohol and head and neck cancer. The alcoholic typically displays cancer of the tongue, tonsils and oropharynx. Alcoholism combined with smoking is the most important cause of cancer in certain sites of the head and neck today (Lowry, 1975).

There has been reported an increase in the IgA concentration of whole saliva and serum in cancer patients above that measured in controls (Brown, Lally, Frankel, Harwick, Davis, and Rominger, 1975; Mandel and Khurana, 1969 and Varsamis, Paraskevas, Averbeck, and Adamson, 1974). Several studies have indicated no consistent marked elevation in parotid saliva IgA levels in these patients (Brown, Lally and Frankel, 1975 and Abelson, Mandel, and Karmioli, 1976). In the studies which showed high whole saliva IgA levels it was suggested that contamination of the saliva with serum occurred due to damaged epithelial mucosa.

We have observed that salivary IgA levels in a control population tend to increase with age. Between age 21 and 41 the slope of this regression line was 0.19 I.U. of IgA/ml/year with a correlation coefficient of 0.69. A negative correlation was observed in alcoholics with the salivary concentration of IgA decreasing at 0.12 I.U. of IgA/ml/year. The correlation coefficient in the alcoholic group was -0.57 .

We have observed increased levels (nearly three I.U./ml) of IgA in parotid saliva samples from young alcoholics. The IgA levels of older alcoholics were lower than those of age matched controls while IgA levels of treated head and neck cancer patients were within the control range. We suggest that the effect in younger alcoholics may represent a significant immune response mechanism. Our results further indicate that this mechanism is significantly reduced in older alcoholics.

These results may be considered preliminary in that salivary flow rates could not be exactly measured. However, flow rates were estimated as samples were collected and individuals with excessively high or low flow rates were excluded from these data. We also have no absolute measures of the amounts of alcohol ingested by either the alcoholic or control groups.

Our results suggest that we were measuring sIgA in parotid saliva and not IgA from serum

contamination. The lack of any measurable IgG in the saliva samples (low level immunodiffusion plates range = 5 to 120 mg/100 ml) which would have been present if serum leakage occurred suggested the IgA measured was not leakage from serum. Mandel and Khurana (1969) have reported that there is an inverse relationship between sIgA concentration and flow rate. Others, however, have stated that although salivary IgA in cancer patients does indeed seem to vary inversely with flow rate, it is always greater in cancer patients than in controls, regardless of flow rate (Brown, *et al.*, 1975). In this study, flow rate wasn't monitored.

It appeared that a decrease in IgA levels was observed after 10 days abstinence of alcohol in the younger alcoholic group. If one hypothesized that the increased levels of IgA in alcoholics was a protective host mechanism, as insult subsides, a decrease in IgA may be observed. Many other variables, however, could be involved in this observed decrease. It is troubling that in the older alcoholic group there was less response to the insult and in fact the IgA levels of this group were less than those of controls.

It was also interesting that less IgA was observed in individuals who displayed greater cigarette consumption. We know that smoking potentiates the onset of cancer in alcoholics and if IgA has a protective function in the alcoholic, smoking, long term alcoholism, and advancing age perhaps allow the individual an even greater risk of cancer.

Lowry (1975) has stated that at least 10 years of alcoholism is needed for oral carcinoma incidence to be positively correlated with alcoholism, and it was interesting to observe lower IgA concentrations in alcoholics of greater than 10 years. Again in a chronically insulted environment low levels of IgA were observed.

It could be suggested that drinking and smoking may induce immunogenic changes or alterations in the host which responds with increased salivary IgA levels. However, it could also be argued that the increased salivary IgA levels which are observed in younger alcoholics presuppose these individuals to oncogenic cell formation.

ACKNOWLEDGEMENTS

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The Dental Hygiene Public Health Concentration

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ABSTRACT

The Public Health Area of Concentration is a recent addition to the Dental School's Baccalaureate Dental Hygiene Program. The Dental Hygiene Department's interest in offering the public health concentration grew from a realization that baccalaureate dental hygiene programs are responsible for designing curricula which help prepare graduates to assume a variety of professional roles. The 1979-80 academic year is the third year that the area of concentration has been in operation. To date, ten students have completed the public health concentration; five of the graduates hold public health positions and one is pursuing an advanced degree in health services administration. The following article describes the curriculum content of the Public Health Area of Concentration.

Four year dental hygiene curricula often provide dental hygiene students with opportunities to develop specific areas of expertise within their profession. The University of Maryland's Dental Hygiene Department has offered its students such opportunities through the development of concentration areas within the Bachelor of Science curriculum. One area of concentration—Dental Public Health—provides the dental hygiene students with experiential and didactic learning situations in the planning, implementation, evaluation and management of community health programs; principles of fiscal management and analyses of health care delivery systems.

The dental public health concentration was introduced into the Bachelor of Science curriculum in the fall of 1977. As originally conceived, the dental public health tract offers specialized learning to a selected group of interested senior dental hygiene students. This year, as in previous years, five students are participated in the program.

The dental public health concentration arose from the recognition of several factors; 1.) that hygienists can and should continue to assume public health roles in the community; 2.) that hygienists may effectively serve as health professionals in settings other than private practice; 3.) that formal education in the dental component of the health care delivery system should be offered to baccalaureate students; and 4.) that the public health component would serve to strengthen and augment the entire curriculum.

Currently, the dental public health concentration consists of eighteen credit hours. Twelve of these hours are considered "core curriculum" and are offered at the dental school. Students take an additional six hours of related course work at University of Maryland Baltimore County or other nearby academic institutions. Related courses include statistics, community development, health administration and relevant psychology and sociology studies.

The first semester core curriculum is composed of two three-hour courses: Community Program Administration (DHYG 415) and the Dental Public Health Practicum (DHYG 416). Community Program Administration's main emphasis is on essentials of effective management and administration.

Areas of management which are emphasized include: women in management, planning, evaluation, decision-making, organization, communication, behavioral science applications in management and grantsmanship. In conjunction with these topic areas, students are involved in assignments and exercises which require the application and synthesis of cognitive principles. Group discussion also promotes affective learning. Other content focuses on principles of fiscal management including budgeting, cost benefit analysis and basic accounting. In-class exercises relate the fiscal information to dental situations and thus enable the students to apply the information to their profession.

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Also during the fall semester, each student plans a public health preventive dentistry program, which comprises the planning phase of the Dental Public Health Practicum. These programs are tailored to meet the needs of a target population at a community site which the student has preselected. The students have considerable latitude in selecting their sites and are encouraged to work with delineated populations of interest to them. As a basis for the practicum planning activities, initial class sessions are devoted to discussions of preventive dentistry, principles of dental public health, the constraints and resources relevant to program development, characteristics of target populations as related to specific preventive dentistry programs and the meanings of key words such as "team," "Community," and "program."

The first semester synchronization of the planning experience of the Dental Public Health Practicum and Community Program Administration enables the students to utilize their newly acquired managerial skills in "real-life" public health settings. From their initial in-person and telephone contacts with the community sites to their data collection visits, the students rely on basic managerial skills of planning, communicating, decision-making and directing.

The planning phase culminates in the development and successful completion of the student's program proposal. This comprehensive report describes all aspects of the proposed program. The major areas which are addressed in the proposal include: community site history, resources and needs; target population demographic characteristics, dental needs and utilization patterns; goals and objectives of the program; rationale for the program (including support from the community and the literature); program methodology and materials; program assessments (on-going and final); and potential problems foreseen and proposed solutions. The program proposal is reviewed by selected dental hygiene faculty as well as the community site representatives. Throughout the planning phase, emphasis is placed on recognition of target population needs and demands, utilization of community resources, the utilization and self-assessment of managerial skills, and the correlation between planning and effective program implementation.

During the second semester in the extramural phase of the Dental Public Health Practicum (DHYG 426), the programs are implemented and evaluated. Program implementation covers approximately a ten week period. A "team" of three to four junior dental hygiene students assist each senior student

in program operation. The utilization of the team heightens the managerial experience of the senior, and provides the juniors with meaningful community based rotations, and more effectively meets the needs of the target population.

This year's public health programs were conducted at the Dickeyville Day Nursery School, the Hannah More Center for emotionally disturbed adolescents, the Frederick County Health Department, the Bernard E. Mason Geriatric Outreach Facility, and the Ft. Meade Military installation. The programs emphasized health education activities. Educational programs and in-service training sessions were developed for target population members, their parents, and in-house staff members. At several of the sites, screenings were conducted to assess need, to provide baseline data for program design, and to serve as a basis for referral.

Upon completion of their practicum experiences, the students conduct the final assessment of their programs. At this time, programs are assessed for actual attainment of goals and objectives; appropriateness of the methodology and materials utilized; acceptance of the program by community site individuals and team members; problems that arose and solutions employed; side effects of the program; and how programs can be revised to ensure further success. Students are also asked to express their personal feelings about all aspects of their programs.

The final three hour component of the second semester, Analysis of Health Care Delivery Systems (DHYG 425) is designed to heighten student awareness of current issues affecting the present health care delivery system. Topic areas of interest include the integration of dentistry into total health care, characteristics of the health care workforce, the role of the federal government in the health care industry, quality assurance, consumerism, and national health insurance. Analyses of other countries' systems are introduced to provide the students with perspectives and insights into current U.S. problems.

In conclusion, the dental hygiene department has cause to be proud of the dental public health concentration area. With the federal government's increasing interest in national health insurance and comprehensive health care facilities, numerous career possibilities in managerial and public health areas await the dental hygienist. Through the dental public health concentration, the University of Maryland is producing a dental hygiene graduate who can be engaged in a rewarding career and who can be an asset to the oral health care delivery system.

A Lack of Life Span Prolongation Following Low Doses of X Irradiation in *Drosophila*

Leslie P. Gartner
Larry J. Granat

ABSTRACT

Drosophila melanogaster were exposed to 0, 4000 and 8000 R of X-rays. The life span of the irradiated populations were not affected by these exposures, suggesting that low doses of ionizing radiations do not prolong the life span of *Drosophila*.

INTRODUCTION

A number of investigators have shown that the life span of insects, as of mammals, is reduced following exposure to ionizing radiations. A major difference, however, lies in the doses that are necessary for life-shortening to manifest itself. Some studies, utilizing even high doses (up to 40 kR) were unable to demonstrate any perceptible alterations of life span (Sacher, 1963; Baxter and Blair, 1967; Blair and Baxter, 1970; Rockford, 1966; Sonnenblick and Gartner, 1967; Gartner, 1970; 1973a; Westerman and Parsons, 1972). Others, however, suggest that doses in the 20 kR range may cause considerable life shortening (Giess and Planel, 1977; DeReggi, 1975), while still others claim that low and very low doses of radiation increase the life span of *Drosophila* (Strehler, 1959; 1962; 1964; Nöthel, 1965; Lamb, 1964; 1965) and other insects (Davey, 1919; Cork, 1957; Tilton, Burkholder and Cogburn, 1966; White and Hutt, 1970; Ducoff, 1975; 1976). Considerable controversy surrounds the low dose effects of ionizing radiation on insect life span (Table I) and radiation-induced increase in longevity has been seriously questioned (Sonnenblick and Grodis, 1963; Blair and Baxter, 1970; Atlan, Miquel and Welch, 1970). The purpose of the present investigation was to examine the effects of low doses of ionizing radiation on adult *Drosophila melanogaster*.

MATERIALS AND METHODS

An Oregon R strain of *Drosophila melanogaster* was housed in a mass bred situation in pint size milk bottles on a cornmeal-molasses-agar medium (Gartner, 1970; 1973b) in a constant temperature cabinet at an ambient temperature of $20 \pm 1.0^\circ\text{C}$. Experimental populations were derived from young parents and were placed in shell vials, five males and five females per vial. Flies were then irradiated on the fourth day of imaginal life with 250 kVcp X-rays (15 mA, HVL=0.94 mm Cu+3 mm Al, added filtration) at a rate of 1332.5R/min for total exposures of 0, 4000 and 8000 R. Subsequent to radiation the flies were returned to the constant temperature cabinet and were monitored daily, five days per week and deaths were recorded by sex. Flies were transferred to freshly prepared media once each week.

RESULTS

The data of the present investigation demonstrates that neither 4000 R nor 8000 R have any demonstrable effects on *Drosophila* imago life span. The unirradiated females of this report had a mean longevity of 90.5 days while the irradiated specimens lived for 90.1 and 84.3 days at 4000 and 8000 R, respectively (Table II). Unirradiated males had a mean life span of 81.0 days, while those exposed to 4000 R lived for 81.7 and those exposed to 8000 R lived for 77.8 days (Table III). Although females had a consistently longer life span than males, the maxi-

Table I. A Compilation of Low Doses of Ionizing Radiations on *Drosophila* Life Spans

Species	Temp.	Type of Radiation	Dose	Dose Rate	Mean Life Span (Days)			Day of Irrad.	Reference
					Male	Female	Comb.		
<i>Dros. mel.</i>									
Att. X-diploid	24°C	250kVX	20krad	775 rad/min		31.3		5	Lamb, 1965
Att. X-diploids	24°C	250kVX	10krad	775 rad/min		25.7		5	
Att. X-diploids	24°C	250kVX	0	775 rad/min		22.9		5	
Free X-diploids	24°C	250kVX	20krad	775 rad/min		19.3		5	
Free X-diploids	24°C	250kVX	10krad	775 rad/min		17.8		5	
Free X-diploids	24°C	250kVX	0	775 rad/min		17.7		5	
Triploids	24°C	250kVX	20krad	775 rad/min		40.3		5	
Triploids	24°C	250kVX	10krad	775 rad/min		41.3		5	
Triploids	24°C	250kVX	0	775 rad/min		36.2		5	
Att. X-diploids	24°C	250kVX	20krad	775 rad/min		31.0		3	
Att. X-diploids	24°C	250kVX	10krad	775 rad/min		26.3		3	
Free X-diploids	24°C	250kVX	20krad	775 rad/min		20.0		3	
Free X-diploids	24°C	250kVX	0	775 rad/min		21.2		3	
Triploids	24°C	250kVX	20krad	775 rad/min		43.8		3	
Triploids	24°C	250kVX	0	775 rad/min		42.0		3	
<i>Dros. subobs.</i>		50kVX	34kR	3713R/min	24.4	84.2		8	Lamb, 1964
B/K hybrid		50kVX	17kR	3713R/min	33.8	112.5		8	
		50kVX	8500R	3713R/min	41.9	106.0		8	Transfer every 4 days
		50kVX	0	3715R/min	46.9	69.5		8	
Ovariless (virgins)		50kVX	34kR	3713R/min		89.4		8	Transfer every 4 days
		50kVX	17kR	3713R/min		110.8		8	
		50kVX	8500R	3713R/min		110.1		8	
		50kVX	0	3713R/min		126.4		8	
virgin		50kVX	8500R	3713R/min		135.5		8	Transfer every 2 days
		50kVX	0	3713R/min		128.8		8	
virgin ovariless		50kVX	8500R	3713R/min		118.5		8	
		50kVX	0	3713R/min		144.1		8	
<i>Dros. mel.</i>	25°C	200kVX	4400R				24.0		Strehler, 1962
<i>Dros. mel.</i>	25°C	200kVX	0				16.5		
<i>Dros. mel.</i>	25°C	100kVX	42kR	1050R/min	11.7	17.3			Nothel, 1965
<i>Dros. mel.</i>	25°C	100kVX	21kR	1050R/min	19.5	24.2			
<i>Dros. mel.</i>	25°C	100kVX	11kR	430R/min	29.5	29.1			
<i>Dros. mel.</i>	25°C	100kVX	8kR	430R/min	33.5	28.5			
<i>Dros. mel.</i>	25°C	100kVX	6kR	430R/min	32.3	26.4			
<i>Dros. mel.</i>	25°C	100kVX	5kR	430R/min	35.7	19.3			
<i>Dros. mel.</i>	25°C	100kVX	4kR	430R/min	35.5	18.7			
<i>Dros. mel.</i>	25°C	100kVX	3kR	430R/min	36.6	18.4			
<i>Dros. mel.</i>	25°C	100kVX	2kR	430R/min	36.6	19.2			
<i>Dros. mel.</i>	25°C	100kVX	0	430R/min	36.5	20.1			
<i>Dros. mel.</i>	22°C	⁶⁰ Coγ	5000R		81.18			3	Atlan, Miquel and Welch, 1970
<i>Dros. mel.</i>	22°C	⁶⁰ Coγ	3000R		82.85			3	
<i>Dros. mel.</i>	22°C	⁶⁰ Coγ	0		90.78			3	
<i>Dros. mel.</i>	22°C	⁶⁰ Coγ	30kR		40			3	
<i>Dros. mel.</i>	22°C	⁶⁰ Coγ	15kR		48			3	
<i>Dros. mel.</i>	22°C	⁶⁰ Coγ	0		58			3	
<i>Dros. mel.</i>	22°C	120MeVX	5kR		82.65			1	Atlan, Miquel and Welch, 1970
<i>Dros. mel.</i>	22°C	120MeVX	3kR		84.16			1	
<i>Dros. mel.</i>	22°C	120MeVX	0		90.78			1	
<i>Dros. mel.</i>	23-25°C	14MeVe-	20krad	1430rad/sec	41.2	40.5		1-2	Mill, Davies, Thompson, Atherton, Lindop and Hollingsworth, 1973
	23-25°C	14MeVe-	9.9krad	1430rad/sec	53.4	46.0		1-2	
	23-25°C	14MeVe-	5krad	1430rad/sec	55.0*	53.0*		1-2	
	23-25°C	14MeVe-	1krad	1430rad/sec	55.2*	53.4*		1-2	
	23-25°C	14MeVe-	0	—	49.3*	53.7*		1-2	
<i>Dros. mel.</i>		⁶⁰ Coγ	25kR	1000R/min	46.16	28.80		4	Giess & Paniel, 1977
<i>Dros. mel.</i>		⁶⁰ Coγ	0	1000R/min	56.91	70.70		4	

Table I. (continued)

Species	Temp.	Type of Radiation	Dose	Dose Rate	Mean Life Span (Days)			Day of Irrad.	Reference
					Male	Female	Comb.		
Dros. mel.		90k VX	4000R	960R/min			47.6	1	Sonnenblick & Grodis, 1963
Dros. mel.		90k VX	0	960R/min			46.2	1	
Dros. mel.		90k VX	4000R	960R/min			46.4	7	
Dros. mel.		90k VX	0	960R/min			44.2	7	
Dros. mel.	25°C	ν	15kR	227rad/min	38.6			1-2	Nelson, 1973
Dros. mel.	25°C	ν	12kR	227rad/min	36.3			1-2	
Dros. mel.	25°C	ν	5kR	227rad/min	38.4			1-2	
Dros. mel.	25°C	ν	0	227rad/min	42.6			1-2	
Dros. mel.		$^{60}\text{Co}\gamma$	16kR	320R/min	24	17		3-4	Henneberry, 1963
Dros. mel.		$^{60}\text{Co}\gamma$	8kR	320R/min	26	19		3-4	
Dros. mel.		$^{60}\text{Co}\gamma$	4kR	320R/min	35	19		3-4	
Dros. mel.		$^{60}\text{Co}\gamma$	0	320R/min	25*	28		3-4	
Dros. mel.	24°C	$^{60}\text{Co}\gamma$	35kR	250R/min	16.5	23			Henneberry, 1967
	24°C	$^{60}\text{Co}\gamma$	28kR	250R/min	18	23			
	24°C	$^{60}\text{Co}\gamma$	21kR	250R/min	21	22.5			
	25°C	$^{60}\text{Co}\gamma$	14kR	250R/min	22	18			
	24°C	$^{60}\text{Co}\gamma$	7kR	250R/min	22.5	22.5			
	24°C	$^{60}\text{Co}\gamma$	0	250R/min	22	23.5			
Dros. mel.	19°C	$^{60}\text{Co}\gamma$	33kR	9000R/min	53.5	67.7	60.2	2-3	Gartner, 1973
	19°C	$^{60}\text{Co}\gamma$	0	—	76.3	78.7	77.0	2-3	
	19°C	$^{60}\text{Co}\gamma$	33kR	9000R/min	43.1	63.5	53.1	2-3	
	19°C	$^{60}\text{Co}\gamma$	0	—	58.0	78.2	68.1	2-3	
Dros. mel.	25°C	250kVpX	25kR	6000R/min	35.6	37.6		1	Blair & Baxter, 1970
	25°C	250kVpX	12.5kR	6000R/min	40.5	45.0		1	
	25°C	250kVpX	0	6000R/min	42.4	51.6		1	
	25°C	250kVpX	25kR	6000R/min	40.9	41.3		1	
	25°C	250kVpX	12.5kR	6000R/min	38.0	42.8		1	
	25°C	250kVpX	0	—	44.0	52.0		1	
Dros. mel.	25°C	$^{60}\text{Co}\gamma$	37500R	1070R/min			42.3	1	Baxter & Blair, 1967
	25°C	$^{60}\text{Co}\gamma$	0				51	1	

* not significant

Table II. Mean Life Span of the Female Populations

Dose	Mean Life Span				Maximum Life Span
	No. of flies	Days	SE	Signif	Days
0 R	47	90.5	4.55		138
4000 R	44	90.1	4.24	NS	134
8000 R	37	84.3	5.03	NS	129

Table III. Mean Life Span of the Male Populations

Dose	Mean Life Span				Maximum Life Span
	No. of flies	Days	SE	Signif	Days
0 R	38	81.0	6.24		138
4000 R	41	81.7	5.84	NS	134
8000 R	33	77.8	5.62	NS	127

Table IV. Mean Life Spans of the Combined Populations

Dose	Mean Life Span				Maximum Life Span
	No. of flies	Days	SE	Signif	Days
0 R	85	85.8	3.75		138
4000 R	85	85.9	3.60	NS	134
8000 R	70	81.1	3.71	NS	129

mum length of life was the same for both sexes at all exposures. The combined data for the two sexes naturally reflect the separated data, again demonstrating that neither 4000 R nor 8000 R decreased the mean life span of *Drosophila* (Table IV).

DISCUSSION

The results of the present investigation strongly suggest that radiation has no beneficial effects on life span in *Drosophila*. The present data, although contradicting those of Lamb (1964; 1965), Strehler (1959; 1962; 1964) and Nöthel (1965) are supportive of many other reports concerning the effects of low doses on fruitfly longevity (Table I). Recent studies of low dose-induced increases of insect longevity suggest that sublethal radiation insults may act as sensitizers and activators of repair mechanism active at molecular levels (Ducoff, 1975; 1976). Although such a repair mechanism has been suggested to be in operation effecting repair at low dose rates and possibly being incapacitated at high dose rates (Gartner, 1970; 1973a) its purported activation by very low doses of radiation is tenuous at best, and is contradicted by a plethora of reports utilizing *Drosophila* (Atlan, Miquel and Welch, 1970; Rockford, 1966; Sonnenblick and Grodis, 1963; Sonnenblick and Gartner, 1967; Gartner and Sonnenblick, 1968; Mill, Davies, Thompson, Atherton, Lindop and Hollingsworth, 1973; Geiss and Planel, 1977; Nelson, 1973; Henneberry, 1963; 1967; Gartner, 1973b; Blair and Baxter, 1970; Baxter and Blair, 1967; Gowen and Stadler, 1952) as denoted in Table I.

Hence, the present results can offer no suggestions as to the *prima causa* of radiation-induced life span prolongation, if indeed such exists. Instead it must argue against any such life span lengthening effects and strongly urge that further studies be initiated to investigate the matter to a greater extent utilizing many other insect species.

SUMMARY

Four day old male and female fruit flies (*Drosophila melanogaster*, Oregon R strain) were exposed to 0, 4000, and 8000 R of 250 kVp X radiation at 1332.5 R per minute. The mean life spans of the irradiated and control populations were not statistically different, suggesting that low doses of X-radiation do not significantly alter *Drosophila* life span.

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The Effects of Hormonal Oral Contraceptives on the Female Human Periodontium and Experimental Animal Models, a Review of the Literature

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ABSTRACT

A literature review of the effects of hormonal oral contraceptives on the female human periodontium, as well as experimental animal models, is presented.

Clinical studies indicate an association between the intake of sex hormones and gingival inflammation. Administration of oral contraceptives has been associated with the production of a pyogenic granuloma, i.e., pregnancy tumor.

Experimental investigations of sex hormone administration reveal alteration of alveolar bone. Gingival tissue regeneration and wound healing may be delayed by intake of sex hormones.

The precise mechanism by which female sex hormones influence the periodontium is unclear. Prostaglandin E₂ has been implicated as a possible mediator of the inflammatory process. It is also suggested that the changes seen in the periodontium represent a circulatory disturbance.

INTRODUCTION

The major etiologic agent responsible for inflammatory periodontal disease is microbial metabolic products. The response of the periodontium to the bacterial plaque may be modified by anatomic factors, restorative dentistry, nutrition, and systemic conditions (1). Changes in the circulating levels of female sex hormones may be associated with changes in the periodontium. Alteration in the levels of these hormones, which occur during pregnancy, puberty, and at menopause, have been implicated as modifying factors in the pathogenesis of periodontal disease. This paper discusses the influence of hormonal oral contraceptives on the health of the periodontium.

DISCUSSION

Characteristic oral manifestations of hormonal contraceptive intake are similar to oral changes associated with pregnancy (2). The most marked clinical feature is the pronounced vascularity of the gingiva. The gingiva is inflamed and exhibits a bright red color. The marginal and interdental gingiva is edematous, pits on pressure, appears smooth and shiny, and is soft and friable. Areas of marked inflammation may bleed easily, elicited by gentle probing. Gingival pain may be an accompanying symptom. Several case reports describing these changes have been reported (3-5). Many clinical investigations studying the effects of hormonal contraceptives have shown an alteration in the gingival tissues in response to the sex hormones.

Studies by El-Ashiry and associates have compared the degree of gingival inflammation for 125 females with respect to length of time of hormonal contraceptive intake (6). To assess the health of the gingiva, the following scoring system was developed:

- 0—No clinically obvious inflammation.
- 1—Slight gingival hyperemia, swelling, loss of stippling; patient unaware of the condition.
- 2—Moderate hyperemia, swelling and loss of stippling; tendency to bleed on pressure; may be tender or painful.
- 3—Marked hyperemia, swelling and loss of tissue tone; spontaneous bleeding; gross ulceration may be present; tender or painful.

The greatest gingival changes were seen during the first three months of contraceptive intake, with a mean gingival score of 1.5, compared to the mean score of the control group of 0.6. During the subsequent three months, there was a further less marked change in the score of the contraceptive users to 1.8. The gingival score tended to decrease after nine months of the hormone intake, so that the score of those taking the contraceptives for one or two years was similar (1.6 and 1.5 respectively) to those taking them for only three months.

Another study by El-Ashiry *et al.*, using similar methodology, compared the influence of pregnancy and oral contraceptives on the human gingiva (7). The gingival score of those women taking the contraceptives for three months was similar to those women pregnant in the first or second trimester. Women taking the contraceptives for six or nine months had scores similar to those women in the third trimester. The gingival score of those taking the contraceptives for one or two years was similar to those women in the first or second trimester. The decrease in gingival scores after nine months or oral contraceptive intake observed in this study, and the similar findings found by the authors in their 1971 study, corresponds to the known clinical improvement in the gingiva after parturition. The similarity of the gingival effects of pregnancy and oral contraceptive intake suggests a common pathogenesis of the alteration of the gingival health.

Knight and Wade, in 1974, reported the effects of hormonal contraceptives on the human periodontium, with respect to length of time of intake plaque scores, pocket depths, and degree of inflammation (8). This study indicated that there were no significant differences in plaque accumulation and extent of inflammation existing between the group of women taking contraceptives and a comparable control group.

The distribution and severity of periodontal disease in women taking oral contraceptives was compared with women not receiving sex hormones in a study by Das *et al.* in 1971 (9). Gingivitis was observed in almost all the patients of both groups. The gingivitis in the control group was predominantly mild and scattered, whereas, the patients taking oral contraceptives exhibited either localized or generalized gingivitis in a significantly higher proportion than the control group. Mean pocket depths measured at different areas of the teeth were also studied. The changes in the pocket depths were varied and inconclusive.

The relationship between gingival inflammation and oral contraceptives has been thoroughly reviewed. The volume of gingival exudate, an indicator of gingival health, has been studied in a group of women by Lindhe and Björn (10). With a combination type of pill, the exudate increased only during the first six months of intake, while with a sequential pill type, increased exudation occurred only after six months of intake.

In subsequent investigations, Lindhe and co-workers attempted to define the role of local and host factors and their relationship to inflammatory periodontal disease. One study by Lindhe *et al.*

observed the influence of increased levels of sex hormones upon gingival exudation in gingivitis-free female dogs (11). For four weeks prior to the hormone administration, all the dogs were supplied with a hard diet. Additionally, once every second day, the teeth of these animals were carefully cleansed with pumice and rubber cups, until no plaque was discernable after staining with the basic fuchsin. Results of this study demonstrated that the gingivitis-free dogs, injected with estrogens or progesterones, were found to have increased levels of gingival exudate. When the administration of these hormones was discontinued, the fluid levels returned to the original volumes.

Further studies by Lindhe *et al.* investigated the effects of repeated administration of sex hormones in dogs with pre-existing chronic gingivitis (12). Results of this study indicated that the intake of estrogens and progesterones seemed to exacerbate the chronic gingivitis, as evidenced by an increased gingival exudate. The tissue response to the hormones seems to indicate that although the gingival inflammation is induced primarily by bacterial plaque, it is aggravated by increased levels of circulating estrogens and progesterones.

Conflicting clinical evidence appears in the literature concerning the extent of local irritating hormonal contraceptives. The study by El-Ashiry *et al.* reported a significant aggravation of the gingiva among women taking contraceptives compared to the control group, despite similar accretion of calculus in both groups (6). Similarly, the amount of plaque present in the study by Lindhe and Björn (10) and Knight and Wade (8) was not significantly different between the control and contraceptive groups; yet, increased gingival inflammation was observed in the hormone groups. Conversely, a study by Das *et al.* revealed that the status of oral hygiene of patients taking oral contraceptives was poorer than those not taking any medication, and this factor explained their poorer periodontal condition (13).

Oral contraceptive intake has been implicated as a possible etiologic factor in producing hyperplastic gingivitis and a pregnancy tumor, i.e., pyogenic granuloma. Two case reports describing these inflammatory lesions have been documented in the literature (3, 14). Discontinuance of the hormonal intake resulted in partial shrinkage of the pyogenic granuloma and a marked decrease in gingival inflammation in one patient (3).

Experimental studies observing the influence of sex hormones on alveolar bone loss have been reported. In a study by Lundgren and Lindhe,

ophorectomized female hamsters, maintained on periodontitis-inducing diets, were injected with estrogen and/or progesterones (15). The control group was given identical diets and received sham operations. Comparisons of the mean bone losses of both groups revealed no significant differences.

Stahl *et al.* studied the effects of estrogens on mice and rat alveolar bone (16). They observed that injections of estrogen led to osteosclerosis of the alveolar bone and appositional bone growth in mice; whereas, in rats there were no significant changes. Similar changes were reported by Shklar and Glickman studying the effects of estrogen on the periodontium of mice (17).

Nutlay and associates also investigated the influence of estrogens on mice and rat alveolar bone (18). In the new-born mice, administration of estrogen led to replacement of hematopoietic marrow of the alveolar process by fibrous marrow, and the marrow spaces were reduced in size by appositional bone growth. The effects on mice older than one year were significantly different. Marked resorption of the interdental and interradicular bony septa occurred in the estrogen-treated mice. Administration of estrogens to rats did not lead to any apparent changes in the periodontium and is in agreement with the other studies.

Presently there are no reports in the literature of the influence of hormonal contraceptives on the health of the alveolar bone in humans. Conflicting data in the animal studies may be accounted for by species variation. The clinical application of these experimental studies remains unclear.

Several experimental studies have reported the effects of gingival tissue regeneration and wound healing in animals treated with sex hormones. Hugoson and Lindhe clinically observed gingival responses following gingivectomies in dogs treated with estrogens and progesterones (19). The authors concluded that the administration of sex hormones did not influence the rate of regeneration of gingival tissue. The position and depth of the gingival crevices were also not affected by the hormone treatment. Despite the fact that there was no differences in rate of recovery of crevice depth, the gingival exudation was significantly greater in the late phase of healing (15-64 days) in the progesterone-treated dogs. Similarly, increased exudation could be seen in the estrogen + progesterone-treated animals 43-64 days following surgery, compared to the corresponding controls.

Hugoson and Lindhe subsequently reported the histological changes found in healing gingival of dogs treated with sex hormones (20). It was

found the estrogen or progesterone treatment did not influence the migration rate of regenerating epithelium, the morphology of the oral and crevicular epithelium, and the amount of fibroblasts and the configuration of the collagen fibers. The only significant finding observed was that the progesterone-treated dogs showed a greater number of polymorphonuclear leukocytes throughout the healing phase, compared to the control or estrogen-treated dogs.

Hugoson and associates also reported the microangiographic changes observed in regenerating gingival in female dogs treated with progesterone (21). The results of their study indicated that although there were no differences between the progesterone and control group in number of regenerating gingival vessels, marked differences were found in the caliber and course of the vessels of the marginal part of the gingival crevices. In the progesterone-treated dogs, rearrangement and differentiation of the newly formed vessels was delayed. In addition, in the progesterone-treated group, several marginal vessels were seen 43 days following surgery which were frequently as dilated and tortuous as those normally found in an earlier phase of healing. The authors concluded that these observations, correlated with the increased exudate levels found in progesterone-treated dogs and the fact that there was a significant increase in the number of polymorphonuclear leukocytes within the crevicular epithelium, suggested an influence of progesterone mainly on the function and permeability of the crevicular vascular plexus of the gingiva.

Numerous experimental studies in animals have attempted to elucidate the mechanisms by which female sex hormones influence the periodontium. Grower *et al.* investigated the biochemical alteration of gingival tissue in response to hormonal contraceptives (22). They studied the "second messenger" c-AMP, a nucleotide which mediates hormonal activity." They assayed c-AMP levels in the gingival fluid of women taking hormonal contraceptives and a control group of women with no history of endocrine disorders. The results of this study indicated a 50% reduction in c-AMP per unit volume of gingival fluid in the women taking the contraceptives, compared to the control group. They concluded that the metabolic changes which contraceptives generated in the gingival tissues were not mediated by changes in the amount of c-AMP present in the gingiva.

The association of female sex hormones and prostaglandins has been investigated. E-type pros-

taglandins have been implicated as potent mediators of the inflammatory process in humans (23). Prostaglandin E₂ levels have been found to be elevated in diseased human gingiva and may contribute to the pathogenesis of periodontal disease (24, 25). An *in vitro* study by El-Attar in 1976 demonstrated that human gingival tissue challenged with estrogen or progesterone significantly enhanced the synthesis of PGE₂ (24). A subsequent *in vitro* study by Holmes and El-Attar in 1977 revealed that increased conversion of estrone to estradiol-17 β occurred in already inflamed human gingiva (26). Increased levels of estradiol-17 β induced stimulation of prostaglandin E₂ synthesis and resulted in aggravation of the inflammatory response.

How oral contraceptives influence vascular changes in the periodontium is unclear. Lindhe and Bränemark studied the effects of female sex hormones and the microcirculation of the gingiva (27). They injected various sex hormones into hamster cheek pouches and found disruption of the periodothelial tissues and inhibition of the migration of mast cells. They also reported vascular impairment, characterized by reduction in corpuscular flow rate and adherence of granulocytes and platelets to the endothelium of the vessel walls. This disturbance may lead to the formation of micro-thrombi and tissue degeneration.

Subsequent experimental studies by Lindhe and co-workers investigated the micro-vascular changes in the cheek pouches of female hamsters following injections of female sex hormones (28, 29). These investigations demonstrated that administration of progesterone and estrogen increased the vascular permeability to a greater extent than estrogen.

Alterations in the human periodontium have been reported associated with intake of hormonal oral contraceptives. Administration of sex hormones may be considered to be either the initiating or complicating factor in modification of gingival health. Proper diagnosis and treatment of the periodontium requires thorough knowledge of the underlying etiology responsible for any apparent deviations from health. As part of every patient medical history questionnaire, the question of oral contraceptive intake should be included.

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Reprinted from Foley's Footnotes:

A Treasury of Dentistry

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One of my favorite byways of research is the almanac. Having read several hundred almanacs, dating from 1733 to the present, I have become convinced that the American almanacs constitute a colorful and revelatory source of information concerning the history of dentistry in this country.

For over a century and a half the almanac entered the majority of American homes—in the cities, in the towns, and on the frontiers. In thousands of those homes, the almanac was the only source of information in regard to methods of preserving health and curing various maladies; therefore, almost every almanac editor felt keenly his responsibility to supply his readers with directions for self-medication. A few selections will, I believe, convince you that the almanac merits inclusive study by the dental historian. From *Hutchins Improved Almanack and Ephemeris* (New York, 1765): "To Cure a Toothache—Let the party that is troubled with the toothache lie on the contrary side, drop three drops of the juice of rue into that side the tooth acheth, let it remain an hour or two and it will remove the pain." [Apparently this advice is based on the theories advanced by Riviere, French physician of the early 17th century.]

From *Poor Will's Almanack* (Philadelphia, 1780): "To prevent the Tooth-Ache, rub the Teeth often with Tobacco Ashes. To clean the Teeth rub them with the ashes of burnt bread." [As regards the use of tobacco ashes as a therapeutic medium, we again encounter a recommendation of Lazare Riviere, given over a century before.

From the 1802 number of the *Hutchins Almanack*: "Persons who have returns of the tooth-ach at certain seasons, as spring and autumn, might often prevent it by taking a purge at these times."

From the *Town and Country Almanac* (Baltimore, 1840): "Remedies for Diseases of the Teeth—If hollowed or decayed, apply compound tincture of Benjamin, or some essential oil, on cotton; chew the roots of pellitory of Spain. Some burn the nerve with vitriolic or nitrous acid, or with a hot iron." [Note the advocacy of Hippocrates' cauterization procedure.]

In *General Taylor's Old Rough and Ready Almanac* (Lancaster, Pa., 1847), under the heading of "A Cure for the Toothache" (When not arising from Rheumatism), the victim is advised to: "Take two parts of powdered alum, and seven parts of nitric aether. One or two grains are to be inserted in the cavity of the tooth, and repeated whenever the pain returns; in a short time the pain will cease to return, and the chemical action which produces the *caries* (decay of the bone) will cease."



I. GENERAL INFORMATION

The *Journal* encourages the submission of manuscripts in the areas of dental research, service, and education.

Two complete manuscripts with illustrations should be sent to the Managing Editor, *The Journal of the Baltimore College of Dental Surgery*, Dental School, University of Maryland at Baltimore, Baltimore, Maryland 21201. The articles which are submitted for publication are expected to follow the format suggested below. It is assumed that the papers are based on original data.

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Each article should be sequentially arranged as follows:

- A. Abstract
- B. Introduction
- C. Materials and Methods
- D. Results
- E. Discussion
- F. Acknowledgements
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References cited in the text should include the author(s) last name and publication year as in "Doe and Brown (1966)." Multiple authorship (more than 2) is initially cited in toto, e.g. Doe, Brown and White (1966). Subsequent reference to the multiple authorship (more than 2) should be made as: Doe, *et al.*, (1966).

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B. Author(s) having two or more publications in a given year should be designated as a, b, etc. Example:

Doe, S. S. and Brown, D. M. 1966a. Heterochromatin in oral epithelial cells. *The Journal*, 20:73-85 1966b. Cytochemical features of oral epithelium. *The Journal*, 20:98-110.

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